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Biomass Conversion

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AN INTRODUCTION TO BIOMASS AND THE BIOREFINERY

WHAT IS BIOMASS?

In its simplest terms, biomass is all the plant matter found on our planet. Biomass is produced directly by photosynthesis, the fundamental engine of life on earth. Plant photosynthesis uses energy from the sun to combine carbon dioxide from the atmosphere with water to produce organic plant matter. More inclusive definitions are possible. For example, animal products and waste can be included in the definition of biomass. Animals, like plants, are renewable; but animals clearly are one step removed from the direct use of sunlight. Using animal rather than plant material thus leads to substantially less efficient use of our planet's ultimate renewable resource, the sun. So, we emphasize plant matter in our definition of biomass. It is the photosynthetic capability of plants to utlize carbon dioxide from the atmosphere that leads to its designation as a "carbon neutral" fuel, meaning that it does not introduce new carbon into the atmosphere. In reality—as discussed later in the description of life cycle assessments of biomass use—we find that biomass fuels are not quite carbon neutral, because somewhere in the life cycle of their production, conversion, and distribution, some fossil energy carbon is released.

Few people understand the scale of energy and organic matter captured by photosynthesis in the form of biomass on the planet. Consider, for example, that the sun sends 3×10^{24} Joules of energy per year to our planet.[1] On average, over the entire globe, plant photosynthesis captures only 0.1 percent of the solar energy bombarding our world, storing 3×10^{21} Joules of energy in biomass annually. How does that compare with world energy production? The U.S. Department of Energy estimates that, in 2000, global

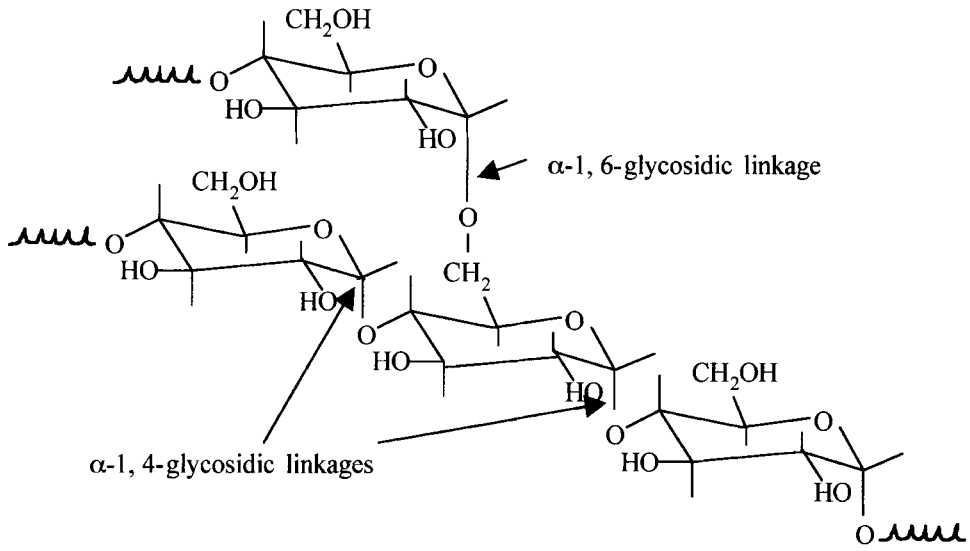


Fig. 33.1. Starch: a biopolymer of glucose molecules used today in the United States as the basis for production of fuel ethanol from corn grain.

primary energy production in the form of oil, coal, natural gas, nuclear power, hydroelectric power, and other forms (including a small amount of renewable energy) was 400 quadrillion Btus annually or 4.2×10^{20} Joules/-year.[2] Thus, energy stored in biomass each year worldwide is seven times greater than humankind's annual energy production. Can we expect to tap all of this energy to meet the ever-growing demand for energy? Could we be taking much greater advantage of this renewable source of organics and stored energy?

The Structure and Composition of Biomass

Why, then, isn't biomass the major source of our energy supply? The answer is that—in comparison to the relatively recent discovery and use of fossil energy sources—biomass has proved more difficult or at least more costly to convert into convenient forms of energy. Thus, although biomass has a very long history as humankind's first primary source of energy, it has tended to find its most important use in modern times as a source of higher-value (societally and economically) food and fiber products.

There are, of course, many forms of biomass. In modern agriculture, many crops are grown for the starch, sugars, protein, natural oils, and fiber they contain. Sugars from sugarcane are used today in Brazil to produce fuel ethanol.[3] They were also the primary souce of fermentable sugars in the United States until molasses became too costly.[4] In the United States today, ethanol made from sugars derived from starch in corn is the largest source of renewable transportation fuel. Starch consists of glucose molecules strung together by α-glycosidic linkages. These linkages occur in chains of α-1,4 linkages with branches formed as a result of α-1,6-linkages (see Fig. 33.1). The ability to release sugars from starch is common to many animals, including humans. It is, thus, no surprise that starch was the first major carbohydrate biopolymer to be used for energy production other than just combustion. Indeed, the use of enzymes to biologically release sugars from starch is one of the earliest examples of modern industrial enzyme technology. It stands as an example of how biotechnology might be used to process other forms of biomass.[5]

To foster a new bioindustry, we need to turn to less used—but more abundant—forms of

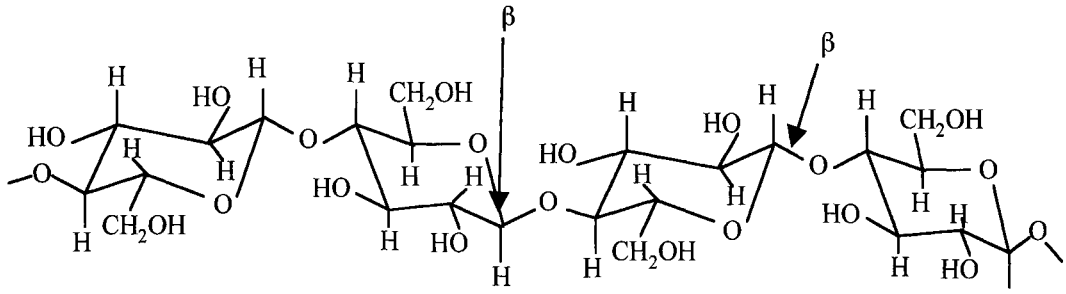


Fig. 33.2. Cellulose: a biopolymer of glucose found in the structural components of all plant cell walls.

biomass. This means focusing on the lignocellulosic components of the cell walls of all higher plants. Here we find highly complex material, both in chemical composition and structure. Plant cell walls are divided into two sections, the primary and the secondary cell walls.[6] The primary cell wall, which provides structure for expanding, is composed of the major polysaccharides and a group of basic glycoproteins, primarily extensins.[7] The predominant polysaccharide in the primary cell wall is cellulose; the second most abundant is hemicellulose; and the third is pectin. Because cellulose is made up only of β-(1,4)-linkages, it has a highly linear structure that encourages the formation of strong hydrogen bonds between chains of cellulose (see Fig. 33.2). The high level of hydrogen bonding among the chains makes it much more difficult to attack or depolymerize, either chemically or biologically. Once depolymerized, however, it is relatively easy to ferment, because it consists of the single sugar, glucose. Hemicelluloses are biopolymers of six- and five-carbon sugars that are almost always branched with a wide spectrum of substituents, including acetyl esters, along the backbone polysaccharide. The more branched and amorphous nature of hemicellulose is more vulnerable to attack, but organisms in nature do not as readily utilize some of its various sugars. Hemicelluloses are thought to hydrogen bond to cellulose, as well as to other hemicelluloses, which helps stabilize the cell wall matrix and renders the cell wall insoluble in water.

The secondary cell wall, produced after the cell has completed growing, also contains polysaccharides and is strengthened by polymeric lignin covalently crosslinking to hemicellulose.[6] Lignin is a high-energy content biopolymer rich in phenolic components. It provides structural integrity to plants. The combination of hemicellulose and lignin provide a protective sheath around the cellulose and this sheath must be modified or removed before efficient hydrolysis of cellulose can occur.

The picture of biomass sketched here offers important insights. The complex structure of lignocellulosic biomass provides protection and structural integrity to biomass. This makes things more difficult for industrial scientists and engineers seeking to convert biomass into useful forms of energy, chemicals, and products. On the other hand, the diversity of biomass composition offers opportunities for processing biomass into a wide range of new and existing chemicals. The challenge to the industrial processor is to develop sophisticated and robust approaches to optimizing the recovery and conversion of each component from this highly complex structure.

CONVERTING BIOMASS INTO ENERGY AND OTHER PRODUCTS

Biological Versus Thermochemical Processing

The distinguishing feature of the various paths from biomass to useful energy is the choice of conversion technology. These technologies fall into two main categories: thermochemical and biochemical conversion. Thermochemical processing, as its name suggests, relies on heat and chemical catalysis to produce useful energy and products from biomass. Likewise, biochemical processing relies on biological organisms and

biological catalysts to transform biomass into energy and products. They are complementary, rather than competing, technology paths that are part of an integrated scheme for making a wide range of fuels, products, heat, and power from biomass.

Thermochemical Conversion of Biomass. The simplest form of thermochemical conversion is direct combustion of biomass to produce heat and power. Direct combustion of biomass for electricity production in the United States currently supports more than 10,000 MW of generating capacity, with the majority of the biomass coming from landfill gas, municipal solid waste, and wood fuel.[8] The Energy Information Administration of the U.S. Department of Energy (DOE) estimates that three gigawatts of electric capacity could currently be supplied by biomass at prices competitive with coal-derived electricity.[9] Direct combustion of biomass in existing power plants is a very effective means of offsetting fossil energy use because it displaces conventional fossil fuels in electric generating stations on essentially a one-to-one basis.

At the heart of most advanced thermochemical conversion processes is the conversion of biomass into simple chemical intermediates in a process known as gasification, as shown in Fig. 33.3. Gasification is a process in which biomass is heated in the presence of air, oxygen, and/or steam to produce a gas mixture that can be used as an energy source itself or can be converted into a variety of fuels and products. Gasification dates back to the early 1800s. It has its roots in technology originally used for the production of gas from coal for lighting and heating.[10] There are many possible variations for the production of a gas mixture often referred to as a synthesis gas or "syngas." The core steps, however, are gas production in a gasification reactor, tar cracking (integrated or separate from the gasifier), and cleanup of the syngas. The clean syngas can then be used to generate power by direct combustion or in a gas turbine/combined cycle system or be synthesized to other fuels or products.

The severity of the conditions in this process affects the distribution of products, which can include pyrolysis oils, chars, and

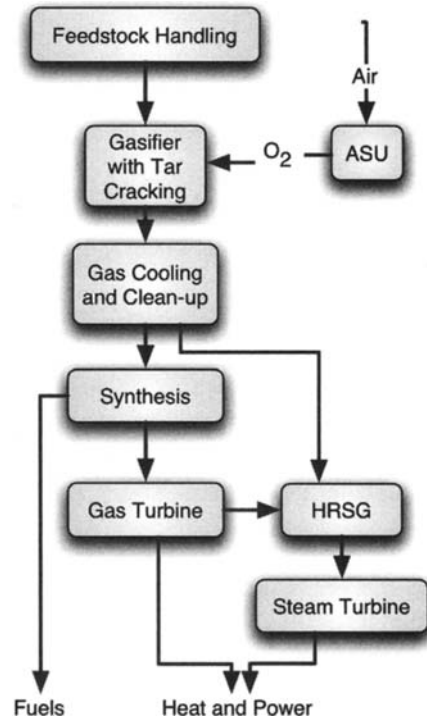


Fig. 33.3. General schematic of a process for thermochemical conversion of biomass to fuels, heat, and power. ASU = Air Separation Unit, HRSG = Heat Recovery Steam Generator.

gases. The chemistry of gasification is relatively simple.[11] The key reaction that takes place in gasifiers is the "water gas shift" reaction. In this reaction, water (provided by steam) can combine with carbon to form carbon monoxide and hydrogen:

$$C + H_2O = CO + H_2$$

The carbon monoxide, hydrogen, and steam in the gasifier can then undergo more reaction to produce more hydrogen, carbon dioxide, and methane. The relative composition of the syngas will depend on the reactor conditions, choice of catalysts, and the relative amounts of air, oxygen, and steam used. A wealth of chemistry is possible with this collection of simple compounds. Syngas can, therefore, serve as a platform for producing all sorts of fuels and other products. Syngas from coal, for example, is used to produce gasoline or diesel fuel products in large-scale commercial operations using so-called "Fischer–Tropsch" catalysts that can

convert these simple compounds into hydrocarbons and oxygenates. Selection of conditions and catalysts in the water gas shift reaction also provides an opportunity to produce hydrogen from biomass for use in fuel cells.[12]

Experience with biomass gasification, however, has been limited to heat and power generation. In the United States, the DOE helped to test gasification of wood, coupled with electricity generation, at a power plant in Vermont, successfully demonstrating this technology at the 200-ton-per-day (5-MW) scale.[13]

Biological Conversion of Biomass. Ethanol is typically the major fuel product of biological conversion. The basic steps of biological conversion are shown in Fig. 33.4. Lignocellulosic biomass can be converted into mixed-sugar solutions plus lignin-rich solid residues by the sequential use of thermochemical pretreatment and enzymatic saccharification. Sugars from hemicellulose and cellulose can then be fermented to ethanol for fuel production. Not shown in Fig. 33.4 is the possibility of biologically or chemically converting these sugars into other products.

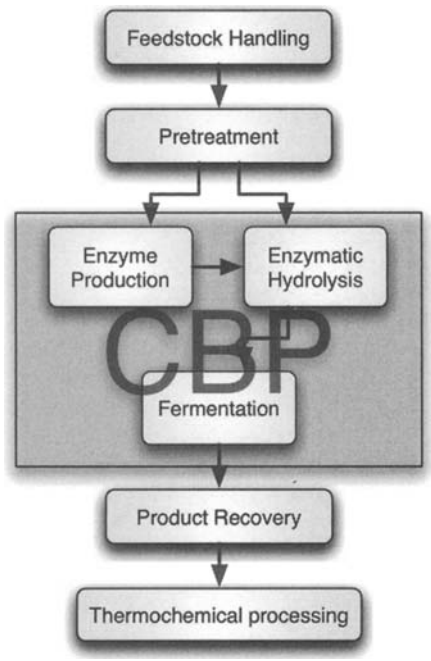


Fig. 33.4. General schematic of a biological process for converting lignocellulosic biomass to ethanol.

There is a long and rich history of using acid and base catalysts to release the sugars found in cellulose and hemicellulose dating back to the discovery of wood sugars in the nineteenth century. The technology was commercialized during World War I in the United States, during World War II in Germany, and later in the twentieth century in the Soviet Union.[14–34] More advanced schemes for biological processing are under development today; however, they rely on this chemical hydrolysis step only as a pretreatment for removal of hemicellulose. Biologically mediated hydrolysis of cellulose is now viewed as the most selective and efficient means of hydrolyzing or depolymerizing the cellulose biopolymer to release its glucose sugar monomers. Many workers in the field agree that cellulose decrystallization and depolymerization are indeed the rate-limiting steps in the conversion of lignocellulosic biomass.

Removal of hemicellulose by dilute-acid pretreatment has been the classic means of rendering biomass more amenable to cellulase action.[35] In a hallmark study, Soltes and coworkers[36] showed that biomass with reduced acetylation responded significantly more favorably to cellulase action than did native biomass. Although still controversial, there is some indication that biomass with reduced lignin content is also more readily hydrolyzed by cellulase action.[37,38] One key to understanding cellulase action on biomass is the fact that the structural and reactive chemical components of the substrate—primarily defined as acetyl and lignin contents—strongly affect enzyme access to cellulose. Another is that once cellulase component enzymes are available in sufficient ratio and concentration at the site of hydrolysis, the degree of cellulose crystallinity controls the hydrolytic rate.[37,39] For the engineer seeking to improve this natural process, the key challenge is to make biomass depolymerization a more rapid and less costly conversion.

To be cost effective, the process must use organisms capable of fermenting the full spectrum of five- and six-carbon sugars released from cellulose and hemicellulose. The advent of efficient genetically engineered organisms equipped with metabolic pathways to handle both types of sugars is an important

improvement in the process that has occurred over the past decade or so.[40–45] The first generation of fuel ethanol technology for lignocellulosic biomass will include the production and use of enzymes for the hydrolysis of cellulose that work with these new versatile fermenting organisms. A future advance in the technology may involve the development of organisms capable of carrying out both the enzymatic hydrolysis of cellulose and the fermentation of the resulting sugars. This configuration—sometimes referred to as consolidated bioprocessing—would represent a significant step toward simplification and cost reduction of the process.[46]

A purely standalone biological process is unlikely. There is a significant amount of residue left over after biological conversion of the carbohydrates. As shown in Fig. 33.4, this residue, containing mostly lignin, is usually sent to some form of thermochemical processing, even if that involves nothing more than direct combustion for production of heat, power or both.

THE BIOREFINERY

The integrated biorefinery is a conceptual framework that capitalizes on the synergies of integrating technologies from both biological and thermochemical process schemes. Furthermore, like the petroleum refinery, the biorefinery has the potential to combine production of low-volume, high-value products with high-volume, low-value fuels or energy production. The ideal biorefinery employs a combination of conversion technologies that maximizes the value of fuels, chemical, materials, and power made from biomass, as shown in Fig. 33.5.

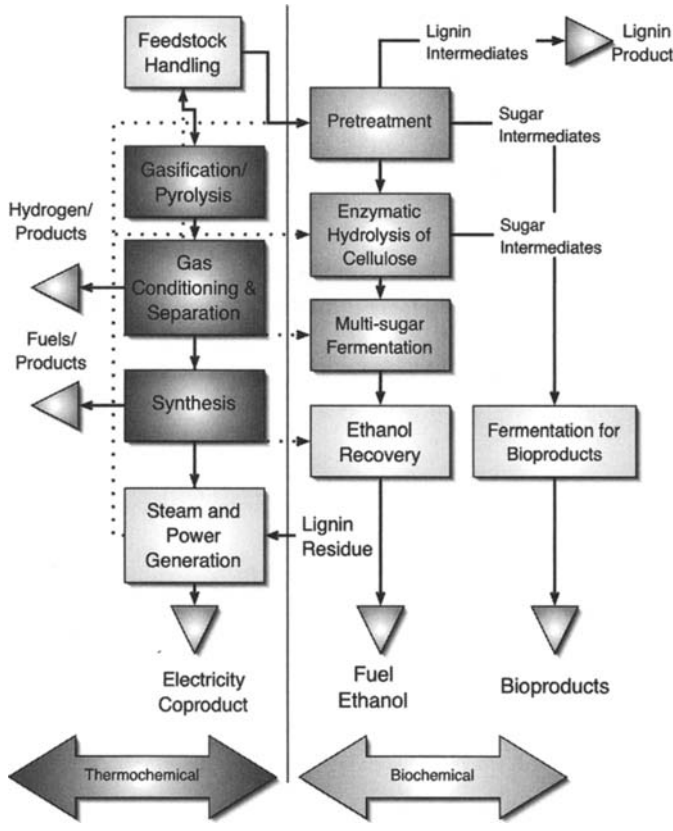


Fig. 33.5. General schematic of an ideal biorefinery combining biological and thermochemical processes for production of fuels, chemicals, heat, and power.

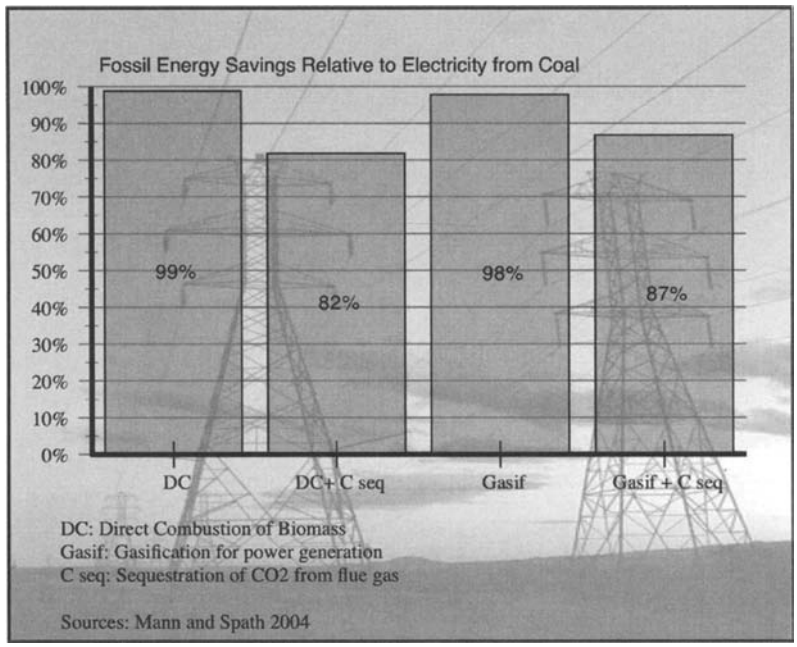


Fig. 33.6. Fossil energy savings for different biomass-to-power scenarios.

The biorefinery should benefit from lessons learned during the evolution of modern-day petroleum refineries. These combine use of fluid catalytic cracking, thermal cracking, and hydrocracking technology to convert the higher-boiling-range fractions of crude oil into more useful lower-boiling-range products. Just as few petroleum refineries use all available conversion technologies, biorefineries too will use only those technology platforms that are most cost effective for converting a certain type of biomass into a certain collection of desired endproducts.

BIOMASS, FOSSIL ENERGY SAVINGS, AND GREENHOUSE GAS MITIGATION

The two most often touted benefits of biomass use are the ability to avoid the use of nonrenewable fossil energy resources and the concomitant ability to reduce net greenhouse gas generation, characteristics that can only be assessed by using life-cycle assessment. Life-cycle assessment[47–52] is an analytical methodology for understanding the full impacts of a given product or service on the environment and on the overall demand placed on our natural resources. In general, this involves looking at all of the stages of production and use of a product or service, from the first extraction of raw materials from the environment to the ultimate disposition of the product. For energy, this includes production and transport of a feedstock (whether that is coal, natural gas, oil, or biomass) to a conversion facility, conversion of the feedstock to a useful energy form, and distribution and use of the final energy product.

Life-Cycle Assessments of Biopower

Figure 33.6 summarizes the results of life-cycle assessments of the impact of various pathways for electricity generation from biomass on fossil energy requirements.[53] Direct combustion and gasification of biomass for power production provide 99 and 98 percent savings in fossil energy use, respectively, compared to combustion of coal for electricity generation. When carbon sequestration is added to either of these options, fossil energy savings are reduced because of the added energy demand for sequestering the carbon

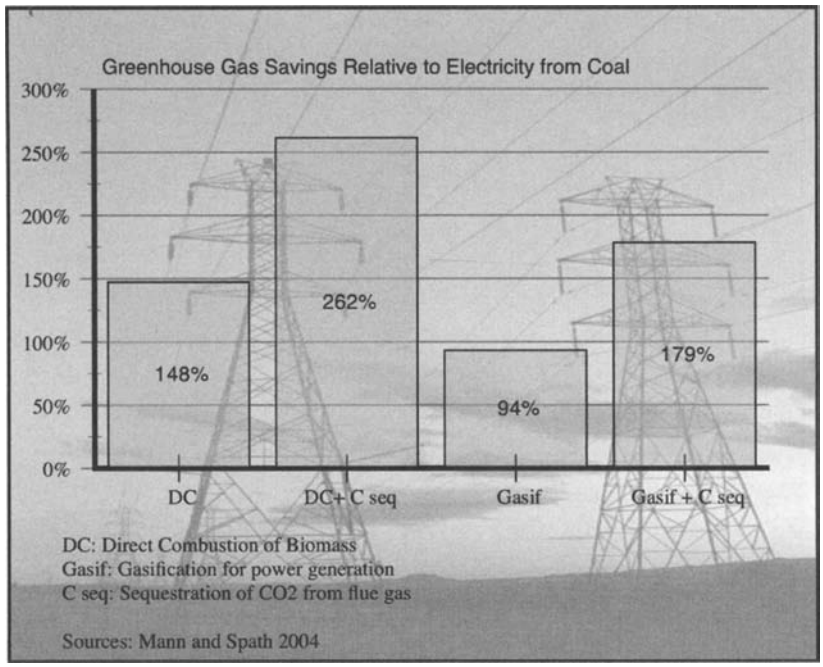


Fig. 33.7. Greenhouse gas savings for different biomass-to-power scenarios.

dioxide from the stack of the power plant. Greenhouse gas savings for these same technology options are also very good, often exceeding 100 percent reductions due to the effects sequestration of carbon in the soil that occurs when energy crops such as switchgrass are grown (see Fig. 33.7).

Life-Cycle Assessments of Biofuels

Figure 33.8 shows fossil-energy savings associated with the production of hydrogen, ethanol, and Fischer–Tropsch liquids from biomass. Savings range from 91 to 102 percent. Ethanol and Fischer–Tropsch fuels are both liquids that can be used in existing internal combustion engine vehicles, whereas hydrogen—which is a gaseous fuel—requires significant changes in vehicle technology, whether it is burned directly for power or used in a fuel cell. Furthermore, although the energy savings for hydrogen are comparable to the other two liquid fuels, these savings may be offset by higher energy requirements for distribution of hydrogen, which are not included in these energy balance calculations.

Although estimates of greenhouse gas emissions for hydrogen and Fischer–Tropsch liquids made from biomass are not available, a number of estimates are available for ethanol made from biomass. These are shown in Fig. 33.9 for the use of E85 (a blend of ethanol, 85%, and gasoline, 15%) in a flexible fuel vehicle. The choice of feedstock can have a significant impact, with corn grain ethanol having the lowest benefits of those studied. The big difference between corn grain and the other biomass resources is that the former relies on fossil fuels to provide energy in the conversion facility, whereas the others make use of residual lignin from the biomass to meet all of the energy needs for conversion, with some left over for cogeneration of electricity.

COST PROJECTIONS FOR FUELS AND POWER FROM BIOMASS

The Competitiveness of Biopower

Figure 33.10 compares the range and midpoint of costs for power production from coal and natural gas with the projected range and

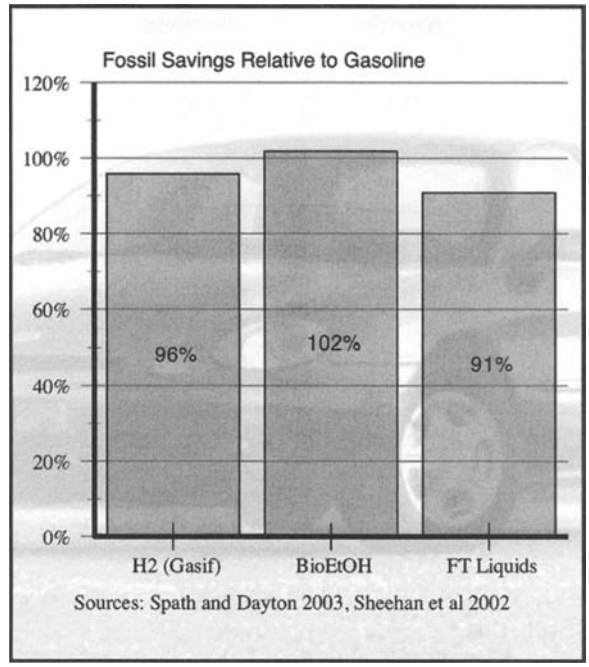


Fig. 33.8. Fossil energy savings for different biofuels.

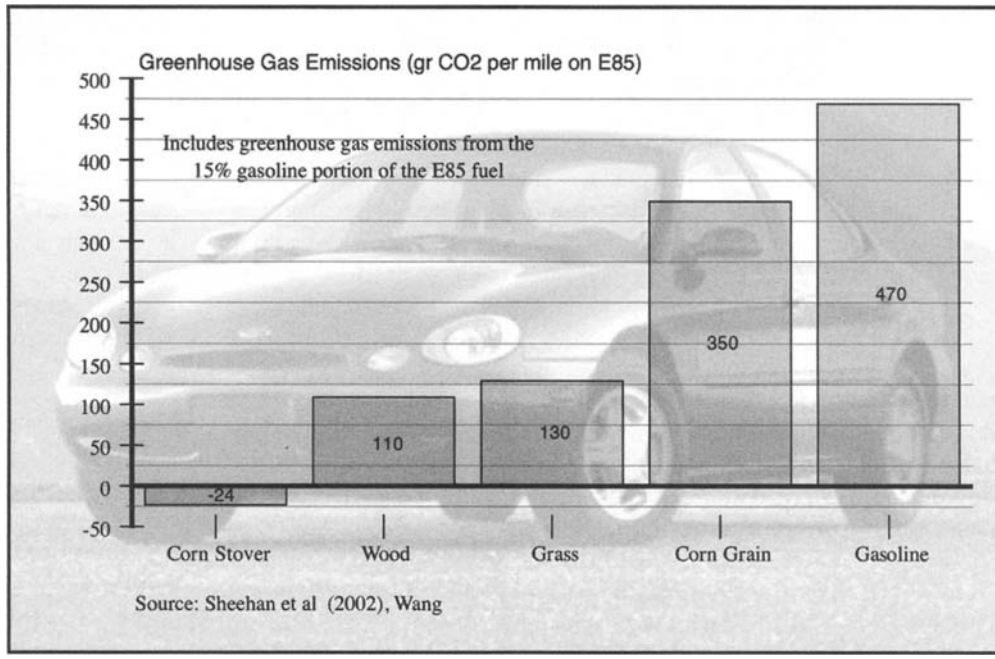


Fig. 33.9. Greenhouse gas emissions for E85 made from different biomass feedstocks and gasoline.

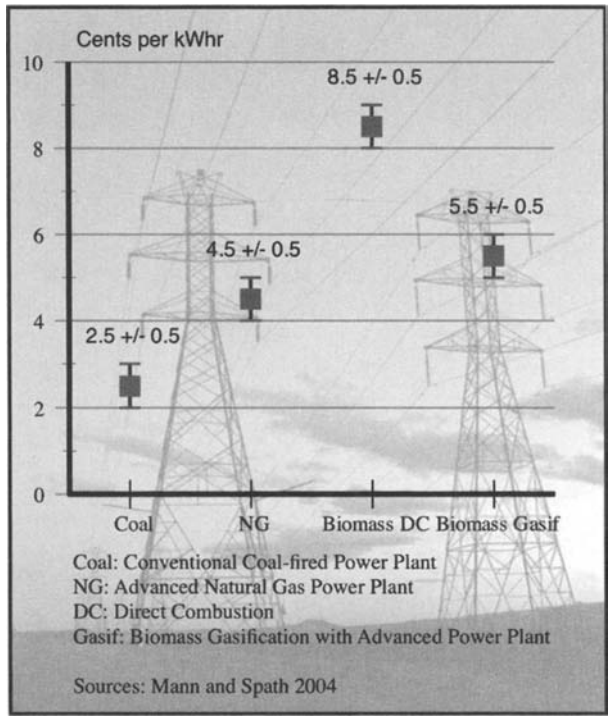


Fig. 33.10. Comparison of costs for conventional fossil fuel electricity generation and biomass power options.

midpoint of costs of several biomass technology options.[54] As the numbers indicate, coal remains the lowest-cost option by far for producing electricity. Biomass technologies are from two to four times more costly and with predictions that coal prices are actually going to decline,[55] this makes the prospects for competitive electricity generation from biomass a goal that will require advances in technology that have not, as yet, even been thought about, much less planned for.

The Competitiveness of Biofuels

Figure 33.11 provides a similar comparison for published estimates of the cost of transportation fuels made from biomass. None of these technologies is ready to compete with gasoline strictly on the basis of delivering energy to a vehicle. In the case of ethanol, the DOE has developed plans for reducing its cost to about $13 per GJ by 2010, which puts this new technology in a competitive range with ethanol made from corn grain, but still out of reach of gasoline (by a factor of two). Recent published long-term (mature technology) estimates of the price that ethanol could reach are hard to find. In the early 1990s, the DOE published estimates for a best-case ethanol cost of $0.67 per gallon, or $8.4 per GJ. In 1996, Lynd reported a "best-parameter" case that brings ethanol cost down to $0.50 per gallon, or $6 per GJ, which is in line with gasoline's wholesale price.[56] These long-term projections show that it is possible for a bio-based fuel such as ethanol to approach parity with its fossil fuel counterpart, but that achieving such parity represents a significant stretch for the technology, with respect to its current or even potential cost.

BIOMASS AS A SUSTAINABLE AND SUBSTANTIAL ENERGY SOURCE

Biomass as a Substantial Source of Energy—Balancing the Demands on our Land

The estimates of global photosynthetic capacity described at the beginning of this chapter

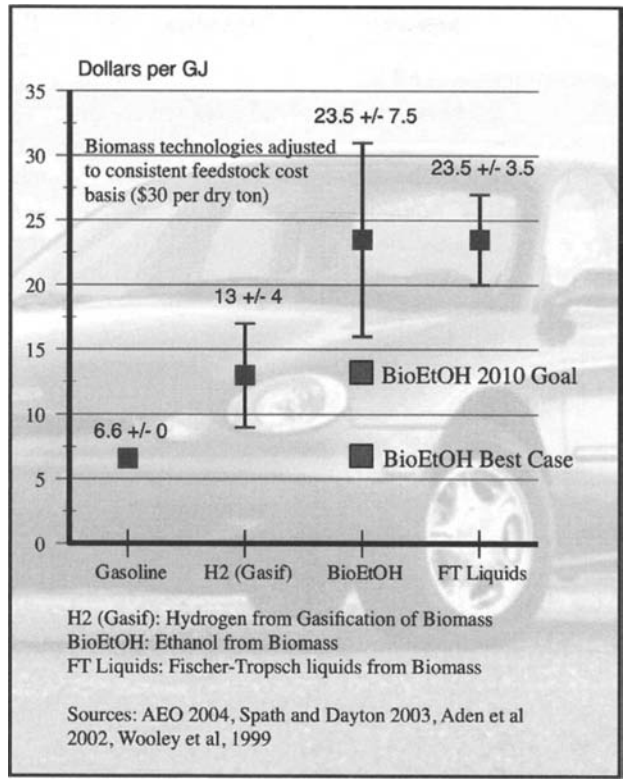


Fig. 33.11. Comparison of costs for gasoline and various biomass derived fuels for transportation.

suggest that biomass could be a truly substantial source of renewable energy. The life-cycle assessments reported in this chapter point to biomass as a highly sustainable source of energy.

The potential of biomass as a large source of fuels and chemicals, however, hinges on how much of the huge global capacity for biomass production can be harnessed for that purpose, after considering other demands placed on our land. And the question has been nothing if not controversial. Why? Because we recognize that the role land plays as the primary source of food and fiber is its most critical job for society. No other resource can provide us with food. So, when it comes to choosing between the use of land for producing food or energy, the choice is pretty obvious. Forget about the use of our land for providing energy to drive our cars and even to heat and light our homes, if it comes at the price of starvation.

Uncertainties of understanding sustainable management of our land, debates about what level of energy use is both adequate and sustainable for our society, and the unknown potential for future advances in science and technology all fuel controversy over the appropriate use of land. The public, policymakers, and even experts in energy find themselves bombarded with widely divergent perspectives. Here are just a few examples of the "pessimists":

- In 2002, a prominent collection of scientists wrote, "Biomass plantations can produce carbon-neutral fuels for power plants or transportation, but photosynthesis has too low a power density (~6 W/m^2) for biofuels to contribute significantly to climate stabilization." These researchers find the scale of our growing energy demand so daunting that they conclude that none of today's current options for renewable energy or energy efficiency are up to the

task. They call for an unprecedented scientific effort to tackle what they call "the technology challenge of the century".[57]

- In 2002, a group of researchers led by David Pimentel dismissed biofuels for transportation outright because of their negative energy balance, and concluded that burning biomass for heat and power might achieve 5.28×10^{18} Joules per year worldwide by 2050. This represents, according to Pimentel's estimates, a 39 percent increase over current bioenergy production over five decades, and only 1.25 percent of world energy output in 2000.

Then, there are the optimists, exemplified below:

- In 2000, an ad hoc committee of leading scientists and engineers established by the National Research Council found that "there is enough unused biomass (in the United States) to satisfy all domestic demand for organic chemicals that can be made from biological resources. . . ." Furthermore, the committee proposed a goal of ". . . eventually meeting over 90% of U.S. organic chemical consumption and up to 50% of U.S. liquid fuel needs with biobased products. . . ."[58]
- Johansson et al. projected aggressive scenarios for a renewable energy-intensive economy in which 40 percent of direct fuel use (for everything except power generation) comes from renewables, mostly in the form of biomass, by 2050. Direct use of biofuels exceeds direct use of oil in their scenario.[59]

Quantifying the controversial and uncertain factors that go into estimating biomass energy potential can shed light on these apparently contradictory findings. Lynd et al.[60] developed a generic equation to describe "R," the ratio of land required for meeting U.S. transportation energy demand with biofuels to the land available for biofuels production. A simplified version is shown below.

$$R = \frac{[\text{Energy Demand}] \times [\text{Land Required per Delivered Energy}]}{[\text{Total Land} - \text{Land Required for Food Production}]}$$

Accounting for all of the factors that go into energy demand (population, vehicle miles traveled per capita, vehicle efficiency) and land required for energy production (biomass land yields, biomass conversion yields, etc.), they found that the numerator in this equation can vary by a factor of 400 for a range of possible high and low values. The denominator can vary tenfold for a range of possible high and low values. This explains the wide diversity of conclusions that has been published regarding the potential for biomass to really affect our energy problem. More important, it points out the need for conducting more rigorous assessments of all these factors. Some of the factors involve social choices, whereas others involve prognostications on future technology developments.

In 2004, several new studies appeared that reconsidered the role of biomass in a future sustainable energy supply. Each has looked at some or all of the above factors, with an eye toward honing in on a more rational assessment of biomass energy supply potential. The Rocky Mountain Institute completed a major study seeking ways of eliminating the United States' dependence on oil. Among five major strategies that, taken together do just that, was an investment in research, development, and deployment of biofuels. They estimated that such a strategy could lead to a 25 percent reduction in our dependence on oil in the year 2025. This corresponds to around 57 billion gallons of gasoline equivalent supplied in the form of ethanol.[61] The Natural Resources Defense Council estimated in its recent assessment of biofuels that around 30 percent of total transportation energy demand could be met with ethanol from lignocellulosic biomass by 2050.[62] This corresponds to about 130 billion gallons per of gasoline equivalent replaced with ethanol.

What sets these reports above others that have been published over the past two decades? First, the organizations themselves are different. They are both nongovernmental

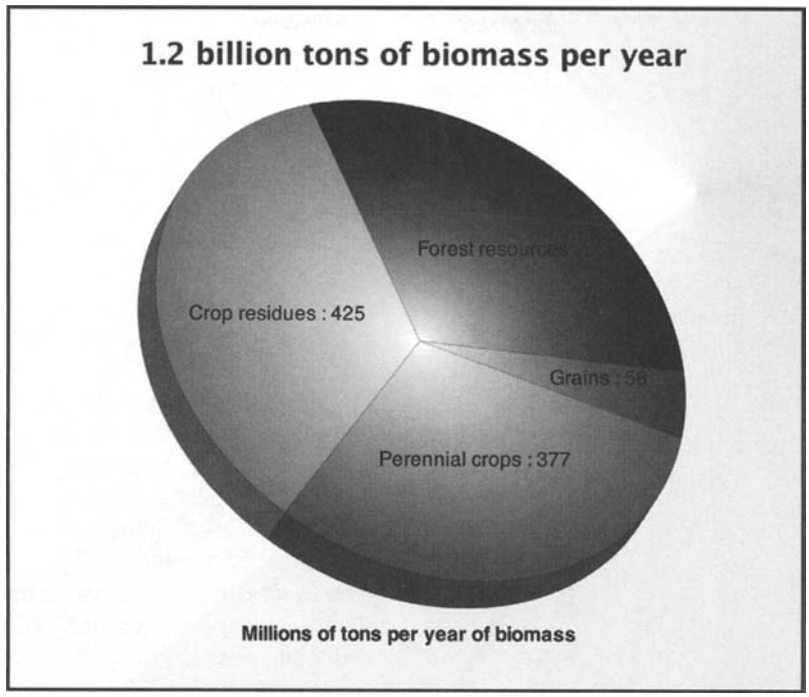


Fig. 33.12. Biomass feedstock potential finds from billion-ton vision study.

organizations (NGOs) that have never been particular advocates of biofuels. That is, they bring the perspective of a "disinterested party" to the table. Second, each looked at biofuels in a rational, future-oriented way. Third, they avoided the "single solution" trap so often found among technology advocates. Their results reflect this. Biomass comes forward as only part of the solution. Gone are the days of single energy solutions, or at least so it would seem from their analyses. Oil was indeed a one-time wonder.

Meanwhile, a third report has been issued in 2005 that looks at the potential supply of biomass from a different point of view. It comes from the U.S. Department of Energy's Office of the Biomass Program and from three offices within the U.S. Department of Agriculture (USDA). These are certainly not "disinterested parties." They are the key organizations in the federal government charged with looking at biomass and the role that agriculture can play in a sustainable energy future. So, it is not surprising that they turned the question of biomass supply on its head. Their starting question: are there a billion tons of biomass out there for conversion to energy and fuels? Or, put a little differently, could biomass replace 30 percent of today's petroleum demand? The motivation for the study was, quite simply, to see if we could prove that there is enough potential biomass to make energy companies and others take a second look at biomass. Figure 33.12 summarizes their findings. They found 1.3 billion tons of plant biomass for the taking each year, or around 80 to 100 billion gallons per year of gasoline equivalent, depending on the efficiency of the biomass-to-fuels conversion.

What can we conclude thus far about the role that biomass can play as a substantial source for our future energy supply? It's worth aiming at. Competing with petroleum-derived energy is still a stretch, but there is still potential for improvement in the technology. As we move forward, we may find disappointments about biomass technology that force us to turn in other directions.

MICROBIAL BIOETHANOL PRODUCTION

The primary carbohydrate components of lignocellulosic biomass consist of D-glucose, D-xylose, L-arabinose, D-galactose, and D-mannose. Glucose (from cellulose) and xylose (from hemicellulose) are the two principal carbohydrates present in most biomass feedstocks. The levels of the minor carbohydrates L-arabinose, D-galactose, and D-mannose (also derived from hemicellulose) vary considerably with biomass type. Softwoods typically contain more glactose and mannose than hardwoods, whereas hardwoods, herbaceous plants, and agricultural residues generally contain higher levels of arabinose and xylose. In some herbaceous crops and agricultural residues, arabinose levels are high enough that conversion of arabinose (in addition to glucose and xylose) is required to achieve overall economic viability.

ETHANOL FERMENTATION SCHEMES

Conversion efficiency and robust fermentation of mixed-sugar lignocellulose-derived hydrolysates are critical for producing ethanol at low cost to realize a commercially viable biorefinery. Biomass sugars are typically released by thermochemical pretreatment followed by enzymatic hydrolysis of chopped or milled biomass. The pretreated soluble fraction of biomass is called the "hydrolysate" and the hydrolysate containing the insoluble material is referred to as the "slurry". In diluted acid pretreatment, most of the hemicellulosic sugars (xylose, arabinose, galactose, and mannose) are solubilized; however, the glucose component remains in the solid form as cellulose, where it is depolymerized by cellulases. This step is often combined with the subsequent microbial fermentation of the sugars to relieve the product inhibition of cellulases, the so-called simultaneous saccharification and fermentation (SSF) process. A process based on the fermentation of pentose sugars (derived from the hydrolysate) combined with the saccharification of cellulose and fermentation of glucose (derived from simultaneous enzymatic saccharification) is referred to as a simultaneous saccharification and cofermentation (SSCF; Fig. 33.13). To be successful, this scheme requires that the microorganisms are capable of fermenting hexose and pentose sugars equally well. Alternatively, a hybrid process with partial enzymatic hydrolysis (to obtain high cellulose hydrolysis rate by operating at high temperature) and co-fermentation may be used to achieve high overall conversion rates of biomass sugars to ethanol. Additionally, microorganisms are often susceptible to inhibitors, such as acetic acid, furfural, and phenolic compounds librated from lignocellulose during chemical pretreatment.[63,64] Because of this, a detoxification step, such as the "overlime process" is generally applied to reduce the toxicity of the hydrolysate.

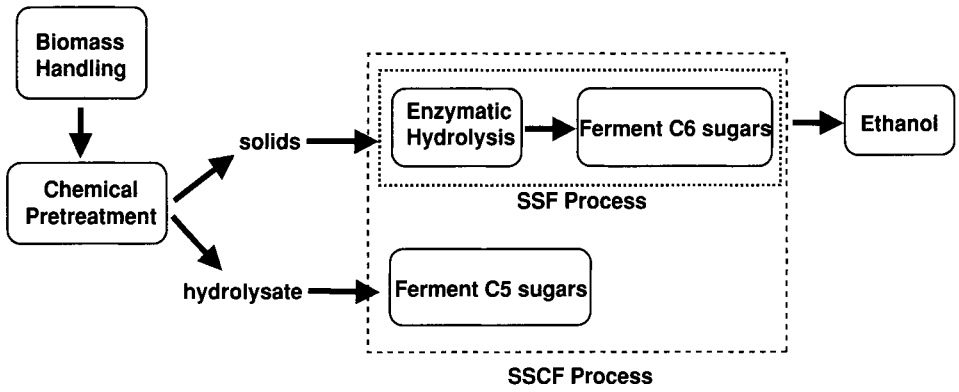


Fig. 33.13. Simultaneous saccharification fermentation (SSF) and simultaneous saccharification and co-fermentation (SSCF) concepts.

Although a number of microorganisms can efficiently ferment glucose to ethanol, only recently has conversion of the pentose sugars in the hemicellulosic fraction become feasible.[65] The few organisms that were known to utilize either D-xylose or L-arabinose typically grow slowly on pentoses and achieve relatively low ethanol yields and productivities.[66] Because of this, the identification and development of microorganisms capable of selectively converting D-glucose, D-xylose, and L-arabinose to ethanol at high yield has been the focus of extensive research during the past 10 to 15 years. In the past decade, the sophistication of molecular biology has grown tremendously and numerous attempts have been made to use recombinant DNA technologies to engineer superior microorganisms for bioethanol production. Only a few of these efforts have been provisionally successful and considerable work is yet to be done.

METABOLIC PATHWAY ENGINEERING

Metabolic pathway engineering is increasingly recognized as a powerful approach for developing microorganisms capable of efficiently converting biomass sugars to ethanol. In broad terms, superior ethanol-producing microorganisms can be developed by either of these metabolic engineering approaches:

1. Broadening the substrate range to include biomass sugars (e.g., xylose, arabinose, galactose, mannose) in strains exhibiting good product selectivity, but not capable of fermenting sugars other than glucose to ethanol
2. Increasing ethanol product selectivity in strains exhibiting broad substrate range

Of course, beyond these two basic approaches, significant metabolic engineering may also be required to stabilize "improved" strains or to enable such strains to achieve high ethanol yields and fermentation productivities.

Following the first approach, *Escherichia coli* and *Klebsiella oxytoca* have been engineered to be highly effective ethanol producers by introducing the genes for ethanol production from *Zymomonas mobilis*.[67–69] Extensive evaluation of these "ethanologenic" strains have been carried out, both in media containing pure sugars and in pretreatment hydrolysates derived from a variety of feedstocks.[68–73]

The second approach, broadening the substrate utilization range of strains that are highly efficient ethanol producers, has been demonstrated by introducing the xylose assimilation and pentose phosphate pathway genes from *E. coli* into *Z. mobilis*. The result was an engineered *Z. mobilis* strain able to ferment xylose to ethnol at high yield.[74–76] An arabinose-fermenting Z. *mobilis* strain was also developed by introducing the arabinose assimilation and pentose phosphate pathway genes from *E. coli* into *Z. mobilis*.[77]

More recently, a long-term effort to develop xylose-fermenting *Saccharomyces* sp. has also been successful. For example, xylose fermentation was reported for *Saccharomyces* strains transformed with the xylose reductase and xylitol dehydrogenase genes from *Pichia stipitis* (additionally, overexpression of native xylulokinase was found useful).[78,79] A recent effort to introduce xylose isomerase from *Pimicus* into *Saccharomyces cerevisiae* was also demonstrated.[80–82] Other noteworthy achievements in the metabolic engineering of superior ethanol producers include initial success in improving the performance of xylose-fermenting yeasts by optimizing the expression of genes encoding the xylose assimilation and ethanol production pathways.[83,84] Successful transformation of the pentose-fermenting *Clostridium thermosaccharolyticum* has also been reported,[85] providing a key tool for further developing this microorganism by altering product selectivity to favor ethanol production. Furthermore, recombinant *E. coli*, *K. oxytoca*, *Z. mobilis*, and *Saccharomyces* were reported to be capable of anaerobically fermenting arabinose to ethanol,[77,86,87] unlike the wild-type xylose-fermenting yeasts, such as *P. stipitis*. These yeasts can grow on arabinose aerobically, but cannot ferment arabinose anaerobically.

PERFORMANCE ASSESSMENT

Efficiency of microorganisms for conversion of biomass to ethanol can be evaluated by three most critical performance factors: yield, productivity, and final product concentrations. Achieving high ethanol yield is the most important factor for the biorefinery, because the cost of feedstocks can be as high as 40 percent of the process cost.[88] Ethanol yield can be referred to either as metabolic yield or process yield. Metabolic yield is calculated as ethanol produced based on sugars consumed, which provides an indication whether microorganisms produce ethanol selectively. The maximum metabolic yields for both heoxoses and pentoses are 0.51 gram ethanol per gram sugars used. Formation of byproducts, such as lactic acid, glycerol, and acetic acid reduces the metabolic ethanol yield, consequently reduces process yield as well. Process yields calculated as "ethanol produced based on total sugars available" provides information not only how efficient the microorganisms can produce ethanol from the sugars, but also the degree to which the microorganisms are capable of utilizing all the sugars available. This parameter is especially important when considering high biomass sugar streams. Similarly, specific ethanol productivity, calculated as ethanol produced per gram of cell biomass per hour, reflects the effectiveness of the catalytic capability of cells. Volumetric ethanol productivity is used to reflect efficiency of the overall process. Kinetic parameters from various recombinant microorganisms were summarized recently by Dien et al.[65]

FUTURE DIRECTIONS

Despite initial success in demonstrating microorganisms capable of fermenting biomass sugars, there is currently a dearth of fermentative microorganisms with the capability to efficiently convert all five biomass sugars in high ethanol yield and productivity under relevant industrial processing conditions. These processes demand robust performance at low pH and high temperature, as well as a high tolerance to ethanol. In most cases, the pentose utilization rate is at least several-fold lower than that of glucose. Therefore, the ethanol yield from pentoses is significantly lower compared to that of glucose.[89]

Unlike the starch-based glucose streams, hydrolysates derived from lignocellulosic feedstocks can contain many toxic compounds that inhibit microbial growth and fermentation.[88] Improving our understanding of inhibition mechanisms and microbial physiology during hydrolysate fermentations will require full use of the advanced analytical and "omics" metabolic engineering and modeling tools recently made available. This approach will greatly enhance our capability to develop a new class of robust industrial microorganisms capable of efficiently and productively converting all biomass sugars to ethanol under "dirty" industrial processing conditions. Other important considerations for commercial viable microorganisms are hydrolysate tolerance and media requirements.

Futuristic process scenarios have been proposed that combine key process steps, thus reducing overall process complexity and cost. One notable example is the consolidated biomass processing (CBP) technology proposed by Zhang and Lynd[90] for the *Clostridium thermocellum* case. Their work reminds us that *C. thermocellum* hydrolyzes cellulose by a different mode of action compared to the classical mechanism associated with fungal-derived cellulases, the "cellulosome." Furthermore, for *C. thermocellum*, the bioenergetic benefits specific to growth on cellulose are result from the efficiency of oligosaccharide uptake combined with intracellular phosphorolytic cleavage of β-glucosidic bonds, another pathway not known in fungi. Zhang and Lynd believe that these benefits exceed the bioenergetic cost of cellulase synthesis, supporting the feasibility of anaerobic processing of cellulosic biomass without added saccharolytic enzymes. Another option for CBP is to enable yeast, already ethanologenic, to produce cellulases.[91] In this case, expression of some active and effective cellulases from yeast has proven challenging;[92] however, endoglucanses and beta-glucosidases appear more amenable to yeast processing.[93]

BIOMASS ANALYSIS AND COMPOSITIONAL VARIABILITY

INTRODUCTION

The Importance of Reliable Compositional Methods for Biomass Analysis

The ability to rapidly and inexpensively obtain an accurate chemical composition of complex biomass feedstocks and biomass-derived materials is a key element in enabling commercialization of processes that convert biomass to fuels and valuable chemicals. Robust analytical methods are needed to improve our understanding of and our ability to economically control biomass conversion processes. Additional challenges face these processes because of the heterogeneity that is an inherent property of biomass. The chemical composition of a biomass feedstock varies as a function of many factors, including plant genetics, growth environment, harvesting method, and storage. Many biomass conversion feedstocks are residues of another process. For example, bagasse is a byproduct of sugar production from sugarcane. In these situations, the varying efficiency in the original process can impart an additional source of compositional variance in biomass feedstocks. All of these sources of compositional variance are difficult if not impossible to control. However, the composition of a given feedstock can be measured at any point and that information can be used to adjust process conditions for optimal conversion or steady-state production. The rapid, inexpensive compositional analysis methods described in this chapter are examples of the types of new tools that will be needed for the commercialization of processes that convert biomass into fuels and valuable chemicals.

As illustrated in Fig. 33.14, biomass feedstocks can vary widely in the number of constituents and the concentration of each constituent. In biomass conversion processes, up to 20 constituents may need to be monitored to characterize the conversion of feedstock into a desired product or products. Standard wet chemical methods for the chemical characterization of biomass feedstocks and biomass-derived materials have been validated through the International Energy Agency and are available from the American Society for Testing and

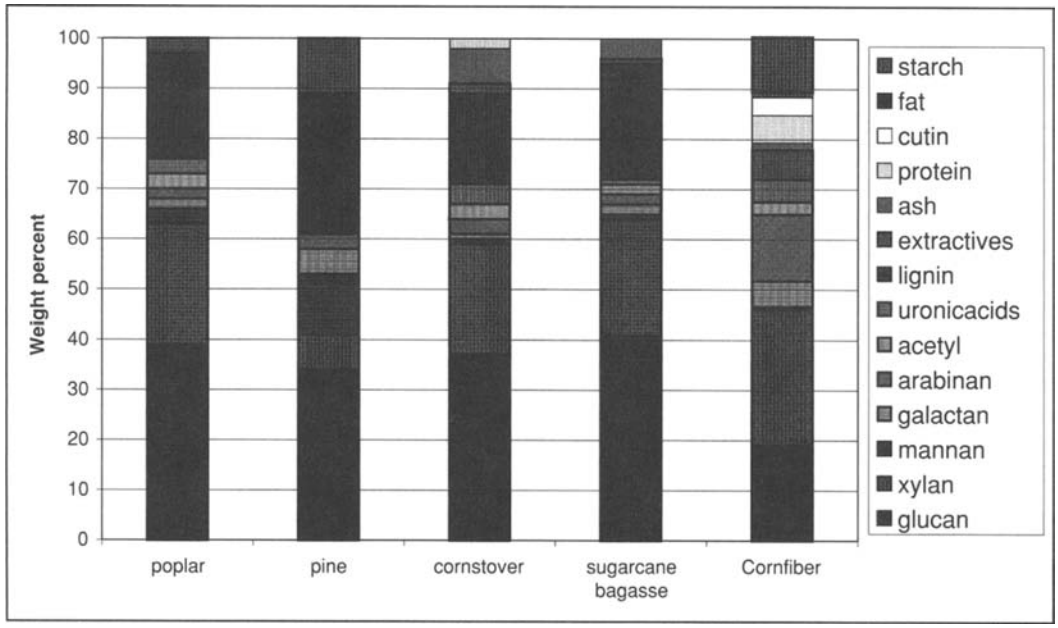


Fig. 33.14. Chemical composition of five commercial biomass feedstocks showing the variance in number and concentration of constituents.

Materials (ASTM).[94] In addition, the National Renewable Energy Laboratory[95] (NREL) has developed and validated a collection of standard laboratory analytical procedures specifically for the compositional analysis of biomass including, but going beyond those of the ASTM. These wet chemical methods of analysis are based on the fractionation of the biomass sample and the isolation of purified fractions that can be quantified using conventional analytical instruments.[96] These methods are primarily used in feedstock-specific portfolios containing analysis methods for each of the relevant constituents. In most cases, these portfolios enable the identification and quantification of greater than 95 percent of the dry mass of biomass feedstock and biomass-derived materials.

The Need for Accurate, Real-Time Biomass Analysis Methods

Standard wet chemical methods, although accurate and robust, are not applicable in a commercial setting, because they are very expensive (labor intensive) and cannot provide the analysis information in a timeframe useful for process control. For example, a complete analysis using standard wet chemical methods costs $800 to $2000 per sample and the results are typically not available for days, sometimes weeks. In contrast, new methods are being developed that can perform the same analysis for about $20 per sample and provide results in a timeframe relevant for process control, meaning that the information can be used to make the process adjustments necessary for steady-state production. One approach to reducing the time and cost of compositional analysis is the development of rapid analysis methods that use multivariate analysis software to extract chemical information from easily obtained spectroscopic data. Rapid analysis methods match the precision and accuracy of their calibration methods, so the savings are obtained without loss of precision or accuracy.[97] New techniques, such as rapid analysis, are needed to provide analytical support for large-scale processes that convert biomass to fuels and chemicals.

Heterogeneity and Biomass Analysis

As stated above, plants are comprised of three-dimensional complexes of natural polymer matrices including cellulose, hemicellulose, lignin, and in some cases protein and silica. These polymer matrices are not uniformly distributed within the plant cell wall, and their relative concentrations change from one morphological region to another, reflecting physiological function. The difference in chemical composition between the tissues within a plant is, in nearly all cases, greater than the variation seen in any one tissue across the entire plant species. An example of this compositional variance is shown in Fig. 33.15 for corn stover from Pioneer Variety 33J56. The concentration of five major constituents is shown for seven tissue types as well as an average composition for the whole plant. The various fractions are plotted according to increasing glucan content. The independent variance of the concentration of various polymer constituents can easily be seen.

Much of the compositional variance in a feedstock can be explained in terms of varying proportions of the tissue types. These proportions can vary by variety, growth environment, harvesting method, and storage conditions. The differences in chemical composition imply structural differences that could also affect conversion efficiency. To understand biomass compositional variability and its implications, it is essential to appreciate the magnitude of variance in available feedstocks and to understand the controlling factors. Appreciating the sources of variance permits the formation of testable strategies for controlling or limiting the sources of variance. This strategy is particularly important in systems where process economics are driven primarily by product yield, as is the case for biomass conversion processes.

Sources of Variance. It is well established in the agronomy literature that factors affecting plant characteristics and crop performance fall into three general categories: genetic, environmental, and interactions between these two classes. In other words, the phenotype (i.e., any measurable characteristic) of an individual plant

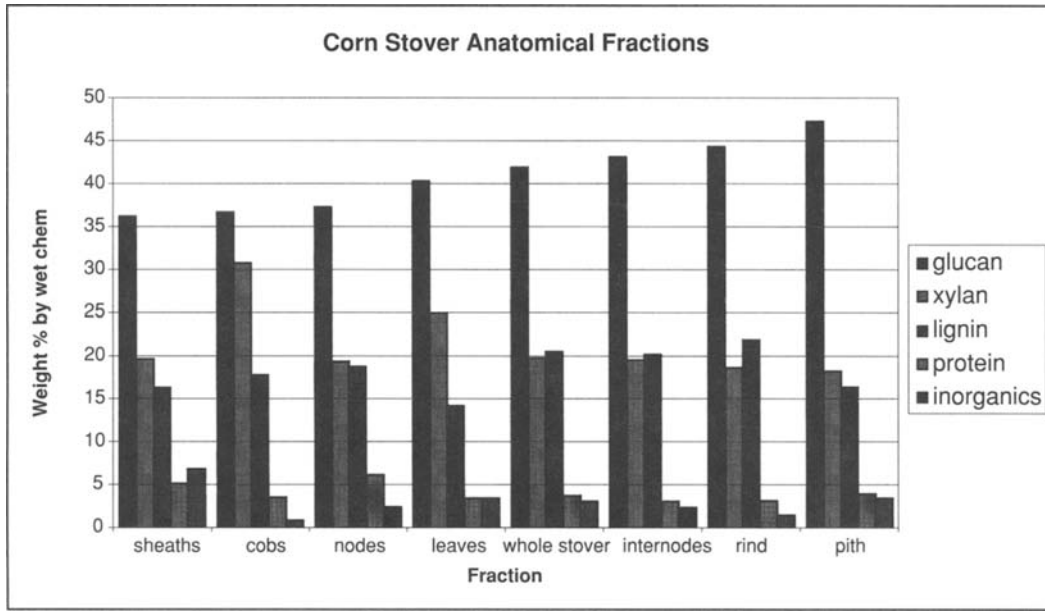


Fig. 33.15. Compositional variability of corn stover tissues from a single hybrid.

is the product of its genotype (i.e., the complete set of genes inherited by an individual from its parents) as influenced by the environment in which that individual exists. For example, the same commercial hybrid varieties of corn often have statistically different yields when grown in different locations (i.e., environments). This may be due to differences between locations in weather patterns, soil types, agronomic practice, or other factors. Similarly, genetically distinct commercial hybrid varieties of corn grown in the same environment often have different grain yields (see results from the Wisconsin Corn Hybrid Trials[98] or the Minnesota Corn Variety Trials[99]). An interaction between these two broad classes of variables is said to occur when different genotypes respond in different ways to a change in environmental conditions.

Agricultural systems are notorious for their variability (e.g., harvest yield) from year to year, location to location, and variety to variety. Characteristics other than yield (e.g., plant height, pigmentation patterns, cell wall composition, nutritional quality, resistance to diseases, etc.) are also influenced by some combination of these and other factors. Each phenotype is most likely influenced by different combinations of genetic and environmental factors.

The analytical methods used to determine the composition of biomass materials are also a source of some variance in this kind of analysis. It must be determined whether variance due to sampling and measurement techniques is significant in light of variance from genetic and environmental sources.

Genetic Factors That May Contribute to Cell Wall Compositional Variability

Modern corn breeding dates back to the early 1900s with the work of Shull,[100] East,[101] and others. Increased grain yield (i.e., productivity) has long been the main objective of corn breeding programs in the United States and elsewhere. Corn breeding programs have produced remarkable results, evident in the historical corn grain yield data for the United States (available online from the USDA National Agricultural Statistics Service Web site[102]) and presented here as Fig. 33.16. It should be realized that the data in Fig. 33.16 reflect both genetic and agronomic improvements made in parallel.

Hallauer et al.[103] present a summary of 13 separate studies that attempt to estimate the fraction of observed corn grain yield improvement

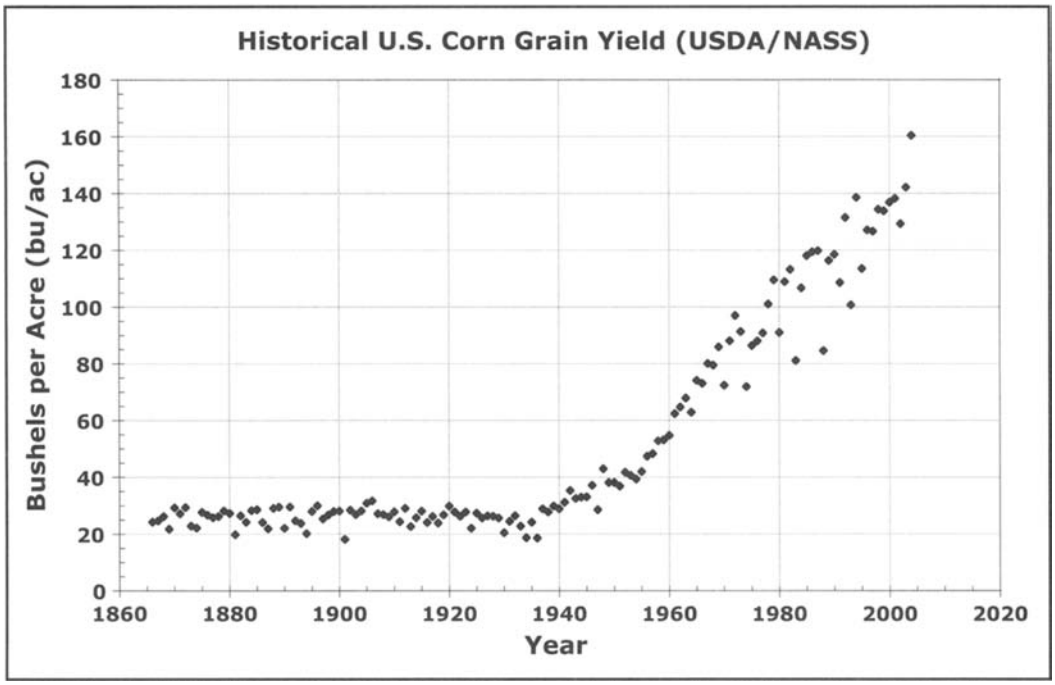


Fig. 33.16. Historical average U.S. corn gain yields in bushels/acre. *(Data from USDA/NASS Web Site.)*

due to genetic gains. These estimates range widely (33 to 89 percent), but it is clear that genetics is responsible for a significant proportion of yield improvement during the past 100 years. The remaining fraction of yield gains is due to changes in cultural practice and crop management (e.g., fertilizer use, higher density of planting, pesticide availability and use, mechanized harvesting).

Peterson states,[104] "Breeders' efforts with maize have uncovered a highly heterogeneous genotype that can be manipulated in most directions to achieve the desired goal." In addition to the overall goal of increased yield, this includes traits such as lodging (i.e., ability to stand), resistance to various biotic and abiotic stresses, male sterility, the angle at which leaves are held relative to the stalk, days to grain maturity, nitrogen-use efficiency, seed composition (i.e., starch, protein, and lipid content), and many others.

Studies relating to the genetic manipulation of nutritional quality (i.e., chemical composition) of corn grain are analogous to this discussion regarding manipulation of stover (i.e., cell wall) chemical composition. This excellent example is used to illustrate what might be accomplished with regard to cell-wall composition using genetic techniques in maize.

The concentration of oil in the kernel varies widely in corn as a species, but corn belt inbreeds range only from 2.5 to 5.5 percent. Commercial hybrids have an even narrower range, typically only 4 to 5 percent. An assortment of breeding strategies starting with outcrossing populations, in which the gene pool is broader than it is for inbred and hybrid cultivars, has altered the oil content of kernels from about 4 percent up to more than 21 percent over a series of selection cycles.[105–108]

A similar story can be told regarding corn kernel protein content. Hybrid corn typically contains 8–11 percent protein, but considerable genetic variability for this trait exists in open-pollinated populations. In an experiment involving serial selection for both high and low protein content, mean protein content has been shifted more than 139 percent from the starting point mean value (a range of at least 20 standard deviations).[105–107] At generation

70 (30 years ago!) in the Illinois Long-Term Selection Experiment, the Illinois High-Protein (IHP) line contained 26.1 percent protein and the Illinois Low-Protein (ILP) line contained 5.8 percent protein. These same lines were simultaneously the low-starch (44%) and high-starch (74.5%) lines, respectively.[109] Thus, at least in corn, selection for increased protein content in grain seems to occur mainly at the expense of starch.

Three plant genomes have now been completely sequenced, and several more are at various stages in the process. From DNA sequence data, it is estimated that the *Arabidopsis* genome contains around 25,500 genes.[110] The rice genome is estimated to contain somewhere between 32,000 and 55,000 genes.[111,112] The *Populus trichocarpa* (black cottonwood), genome is estimated to contain about 58,000 genes.[113] To date, sequencing efforts in *Zea mays* (i.e., maize; corn) indicate that its genome also contains about 58,000 genes.[114]

Cell wall biogenesis during cell growth and differentiation involves many different enzyme activities and perhaps several thousand genes. It has been estimated that 15 percent of the *Arabidopsis* genome (i.e., more than 3800 genes) may be dedicated to cell-wall biogenesis and modification.[115] Although only a very few of these genes have been identified and characterized to date, several efforts are underway to do so (for example,[116–118]). Because multiple alleles (i.e., gene variants) probably exist for each of these genes, the possible number of permutations of genes that affect cell walls in a single species is truly enormous.

According to Carpita,[115,116] genes involved in plant cell-wall biogenesis fall into one of six functional categories:

- The synthesis of monomer building blocks of cell-wall polymers, such as nucleotide sugars and monolignols
- The biosynthesis of oligomers and polysaccharides at the plasma membrane and ER-Golgi apparatus (i.e., polysaccharide synthases and glycosyl transferases)
- The targeting and secretion of Golgi-derived materials
- The assembly and architectural patterning of polymers (includes glycosyl hydrolases and structural proteins)
- The dynamic rearrangement of wall polymers during cell growth and differentiation (includes lignification)
- Signaling and response mechanisms

Although the metabolic pathways associated with the first category have been pretty well elucidated at this point, the enzymes involved in the subsequent categories are largely uncharacterized. Likewise, most of the corresponding genes are also unknown. Significant progress has been made in some of these areas in recent years, however.[119–123]

Once identified, those genetic factors that exert a large influence on cell-wall composition, cell-wall architecture, or biomass conversion process performance characteristics could become targets of a plant breeding strategy to manipulate these characteristics, while maintaining or increasing traditional crop yield or overall agronomics. To get the most from such a breeding program, it will be essential to monitor the effect of a wide range of environmental variables on genetic potential during the breeding process.

Environmental Factors That May Contribute to Variance

Environmental factors that affect plant phenotypes fall into two categories: namely, those that are at least partially under control of the farmer and those that are not. Factors that are largely not under human control include weather patterns (day length, solar flux, temperature, and precipitation), soil type and pH, and soil mineral content, and are not discussed further. A partial list of factors that can be addressed by the farmer include selection of hybrid variety, planting date, harvest date, tillage practice, irrigation, fertilizer types and amounts, pesticide and herbicide usage, and the strategy employed for harvesting, transporting, and storing crop residues. Which of these factors are most influential across the broadest range of situations with regard to stover compositional variability is an open question and will require

TABLE 33.1 Confidence Intervals for NREL and ASTM Standard Wet Chemical Methods

Constituent	*Method Variance Absolute Error 99% Confidence*
Glucan	1.5
Starch	1.0
Xylan	1.5
Arabinan	1.5
Mannan	1.5
Galactan	1.5
Uronic acids	1.5
Pection	1.5
O-acyl groups	0.5
Structural inorganic	0.5
Sucrose	1.5
Protein	1.0
Lignin	1.0
Ferulic acid esters	1.0
Extractives	1.0
Phytate	0.5
Cutin	1.5

multiyear, multilocation field trials to determine with confidence.

Most important are likely environmental factors associated with cultivation, crop management, and postharvest processes that can be controlled to some extent and on the other hand also have a large impact on the chemical composition, cell-wall architecture, or conversion processing behavior of plant biomass. These factors could form the basis of a testable strategy to decrease the variance associated with these characteristics.

Analytical Variance

A complete analysis characterizing greater than 95 percent of a biomass sample can require up to 30 independent measurements to report the concentration of 10 to 15 constituents. Table 33.1 shows the confidence intervals for the standard wet chemical methods used to characterize biomass feedstocks. The confidence interval of 1.5 percent for glucan means that when a value of 38 percent is reported for a biomass sample, there is a 98 percent probability that the "true" glucan concentration lies between 36.5 and 39.5 percent. Many of these values were determined in an international round robin evaluation of four biomass standard reference materials available from the National Institute of Standards Technology (NIST).[124]

PORTFOLIO METHODS

In the past, efforts were made to develop universal methods of analysis that could be used for any type of biomass or biomass-derived material.[125] These methods provide instruction for the various procedural steps, but they rarely discuss why a given step is included in the procedure. The assumption is made that all steps in a standard procedure will be followed as written without omission or amendments. When combined for a complete mass analysis, many analytical methods were compromised to the extent that they were adequate for most samples, but optimal for none. As the biomass conversion industry matures, the need for more accurate and precise methods will increase, as data generated from these methods will be used to evaluate a maturing state of technology for commercialization. In response to this need, newer methods of analysis are being developed in a modular fashion and designed to be incorporated into sample-specific portfolios capable of providing a customized total analysis of any biomass sample. In the portfolio, the individual constituent methods are independently validated relative to standard reference materials. These methods are then validated a second time when the results are combined to reconstruct the entire chemical composition of a biomass sample. Typical mass closures between 96 and 104 percent confirm that no major components have been overlooked and interferences between methods and double counting of materials are minimized.

Wet Chemical Methods

Within each portfolio, the sequence of application of the various analytical methods is critical, as many methods have been validated with the assumption that all prior steps have been followed. For feedstocks, the sequence is sample preparation, then removal of nonstructural

materials through solvent extraction, followed by hydrolysis of the structural polymers to simple forms for chromatographic or spectroscopic analysis. In the past, many methods for biomass analysis have been "behavior based," meaning they define chemical structure based on solubility in certain solvent systems. The newest methods incorporate modern analytical instruments, which allow the various structural components to be measured and tracked based on chemical structure.

Before using biomass analysis methods, analysts are encouraged to review the literature that supports the standard methods.[125] Understanding the science behind the methods helps the analyst understand the ways that the various procedures are connected, the limits within which a step can be altered or modified, and the potential downstream consequences of omitting any given step. With this in mind, the following several paragraphs outline available information concerning the significance, implications, and limitations of each step in a complete characterization of biomass. More detail is available in the open literature and the text of the individual procedures.

Sample Preparation. Sample preparation is an important but often overlooked part of biomass analysis. Biomass sample preparation must take several factors into account. The object of sample preparation is to enable the analysis to be done on a small scale while ensuring that the analysis of the small sample provides valid information about the larger bulk sample. Typically, biomass sample preparation includes drying, milling, sieving, and homogenizing.

Drying. Biomass feedstock samples can contain as much as 60 percent (w/w) water. If the biomass has been degraded in a manner that disrupts the ultrastructure of the plant (chemical treatment or enzymatic digestion), the moisture of the sample could be even higher. Water in a biomass sample, if high enough, can introduce errors during some of the analysis steps by diluting reagents. Moisture in samples can also cause sample fractionation during milling and sieving. Biomass samples with high moisture contents are still vulnerable to biological degradation and may not remain stable and uniform for the duration of a battery of tests. For these reasons, biomass samples are usually dried until the moisture content is less than 15 percent. Because some of the constituents are heat sensitive or volatile, certain protocols must be followed when drying a biomass sample for compositional analysis, to avoid sample loss or degradation. Air-drying is preferred if time, space, and ambient humidity will allow the sample to reach a moisture level below 15 percent. Drying the samples in a convection oven or vacuum oven where the temperature is not allowed to exceed 45°C is an acceptable alternative. When freezing does not cause collapse of the cellular structure of the biomass, samples can also be safely lyophilized, or freeze-dried.

Washing. If the biomass sample is contaminated with soil, the samples should be washed if possible before further processing. Soils vary considerably in chemical content and even a few weight percent of extraneous inorganic compounds can neutralize reagents, catalyze side reactions or affect subsequent analysis in ways that are difficult to observe or control. Washing may be as simple as agitation in clean demineralized water or may require small amounts of mild detergents to be effective. After washing, the sample must be carefully dried before analysis.

Milling. Size reduction is an important step in biomass analysis because standard methods are optimized and validated for materials with a specific particle size. The particle size will affect hydrolysis or digestion rates as well as rates of degradation and should be specified in any standard procedure. As a general rule, the sample to be analyzed should contain at least 100 particles of the biomass sample in order be representative of the bulk material. For reasons described earlier for drying protocols, care must be taken during the milling process to avoid heating the biomass sample. This is particularly crucial if the biomass must be reduced to a fine powder. Cryo-mills, where the milling apparatus is submerged in liquid nitrogen, or mills that are jacketed for the circulation of cooling solutions are most suitable for milling

in these situations. Knife mills such as the standard Thomas–Wiley Mill 4 (Thomas Scientific Model 3375E15 or equivalent) work well for most woody biomass samples. Less dense materials such as corn stalks or straws may be easier to feed into a hammer mill. Most knife and hammer mills will retain and reprocess materials until they pass through a screen of a particular particle size, allowing the user to specify the maximum particle size of the processed sample.

Sieving. All forms of chemical or thermal hydrolysis perform better when the particles are of a uniform size. For this reason, most standard procedures usually specify both a maximum and a minimum particle size. To accomplish this, the milled biomass is sieved through a set of standard screens. To prevent errors associated with incomplete hydrolysis, the oversized material is milled again until it passes through the largest screen. The material that passes through the larger screen but is retained on the fine mesh screen is of uniform particle size and can be used for compositional analysis. The material that passes through the fine mesh screen may be hydrolyzed too quickly leaving the hydrolysis products more susceptible to side reactions or degradation before analysis. Because the fine material cannot be reprocessed to increase particle size, this material must be discarded. The fine fraction should always be removed from the analysis if it has significantly higher ash content than the uniformly sized material. This elevated inorganic content may indicate the presence of nonbiomass contaminants (typically soil).

The impact of extraneous inorganic matter on the analysis cannot be predicted. Most chemical analysis methods have been optimized for bark-free wood that has an ash content of less than 2 percent. These methods may not be applicable to herbaceous materials where structural silica can increase the ash content to more than 15 percent of the dry weight of the biomass. One potential problem with high ash samples is that inorganic materials, depending on their composition, may neutralize the sulfuric acid solutions used in the hydrolysis steps. The affect of pH changes on the hydrolysis of the carbohydrate polymers is unknown, but could result in incomplete hydrolysis of some carbohydrate structures. It is also possible that soil components such as iron and manganese could catalyze undesired side reactions, which produce products that would not be identified and quantified with the current analytical methods. However, although the impact of soil contamination cannot be easily determined, removal of the –80 mesh fraction can improve the chemical analysis by reducing the ash contents of samples to about the level of structural inorganics expected in that type of biomass. The fine material is weighed, reduced to ash to determine the percentage weight of inorganic material and then discarded. The analysis is performed only on the intermediate, uniformly sized material. The composition of the whole sample can be calculated by assuming that the biomass portion of the fines has the same chemical composition as the fraction that is analyzed. Although this assumption is not completely accurate, the precision gained by removing the fine material more than compensates for the error introduced by this assumption. If the sample has not been washed, removing the very fine material (less than 80 mesh) will remove most of the dirt entrained in the sample.

Making the particle size as uniform as possible also makes it easier for the analyst to select a small but reproducible sample for analysis. In some biomass samples, however, sieving to remove fine material chemically fractionates the sample. This is known to be true with wood that contains a significant amount of bark. The bark tends to granulate during milling and will preferentially be removed with the fines. The sample to be analyzed would then have an artificially low bark content relative to the original sample. A similar problem has been reported with some grasses where the friable pith material may be preferentially removed as fines. These samples should not be sieved for fines removal. Samples should be examined for soil contamination, and special care should be taken to select a representative particle size distribution for analysis.

EXTRACTIVES DETERMINATION

Plants store nonstructural materials in their vessels and water transport channels that can interfere with accurate chemical compositional analysis. These materials are commonly known as extractives, which may give the impression that they are a single substance or at least a few closely related substances. This is not always the case. Extractable materials include gums, resins, pitch, waxes, sterols, flavinoids, tannins, terpenes, quinones, nonstructural sugars, chlorophyll, and many other minor building block reserves that vary seasonally and by biomass type. Because extractives vary so much in chemical composition, their behavior during the slate of analyses required for a compositional characterization cannot be predicted. For this reason, nonstructural components should always be removed prior to analysis for lignin or carbohydrates. Failure to remove these materials can cause several problems.[124] Some of the extractives may be insoluble in acid, will precipitate, and thus will be falsely counted as lignin. Because of changes in the physical properties of the extractives, the presence of some materials will limit access to the carbohydrate polymers. If extractable ash, starch, or protein is present in the biomass sample, separate measurements must be made to remove the contribution of this material from the extractives values and to report it more accurately in the correct constituent category.

Carbohydrate Determination

Most analysis methods for the determination of carbohydrates in biomass incorporate a two-stage acid hydrolysis to separate individual polymers and hydrolyze them to simple compounds that can be readily analyzed by chromatographic or spectroscopic techniques. The first stage subjects the biomass sample to a concentrated acid that disrupts the noncovalent interactions between biomass polymers. A second, more dilute stage follows, which is optimized for complete polymer hydrolysis and minimized degradation of monomeric sugars. Failure to remove nonstructural materials may result in incomplete hydrolysis of the more recalcitrant carbohydrate polymers, particularly glucans and galactans. Increasing the hydrolysis severity may result in excessive degradation of the accessible sugars. Hemicellulose sugars, especially xylans and arabinans, are the most susceptible to degradation if hydrolysis severity is increased. If nonstructural sugars are present in the biomass, they should be removed and quantified during the extractive procedures. If both starch and cellulose are present, they will both contribute to the measured glucose concentration after hydrolysis. An independent starch measurement allows the two polymers to be accurately quantified. Some degradation of sugars is unavoidable in the two-stage hydrolysis methods. Carbohydrate standards of similar structure and concentration should be run in parallel to assess the magnitude of this degradation and correct the reported values for this loss if necessary. Structures such as uronic acids, ferulic acid esters, and *O*-acetyl groups are substituents on the hemicellulose polymers and should be included in a complete carbohydrate analysis.

Lignin Determination

Most lignin methods are still based on behavioral definitions: lignin is most often defined as the material insoluble in acid after hydrolysis of the carbohydrate fraction. This assumption is invalid for many biomass samples, particularly herbaceous materials where protein will condense with the lignin. Structural inorganics will also partition during acid hydrolysis with an irreproducible portion remaining in the acid-insoluble residue. Additional determinations must be made on the acid-insoluble residue to convert the gravimetric measurement into a valid lignin measurement. Failure to make these corrections results in falsely high mass closures, as protein and silica would be measured in more than one constituent category. In the two-stage hydrolysis, some acid-soluble lignin may be released into the hydrolysis liquor. An accurate lignin value includes a measurement of both acid-soluble and acid-insoluble lignin. Acid-soluble lignin is traditionally measured by UV/VIS spectroscopy. In these determinations,

care should be taken to ensure that the measured absorbance is made in a concentration range where detector response is known to be linear and that an appropriate wavelength and absorptivity is selected. The references standard method portfolios include options for several biomass categories.

Protein Determination

To estimate crude protein content of biomass or other materials, the nitrogen content of the material is measured by Kjeldahl or combustion methods and multiplied by a conversion factor where:

Protein (wt/wt%) = Nitrogen (wt/wt%)
* Nitrogen-to-Protein Conversion Factor

A nitrogen-to-protein conversion factor (*N*-factor) of 6.25 is commonly used for animal feeds and other materials. The practice of using 6.25 as an *N*-factor is based on an incorrect assumption that protein in a given material contains 16 percent nitrogen (100/16 = 6.25).[126]

The correct *N*-factor for protein found in herbaceous biomass will likely be different than 6.25. Yet, determining a perfectly accurate *N*-factor for the complex matrices of biomass feedstock and process samples may not be possible. The challenge is then to determine the most accurate *N*-factor possible for biomass feedstock and process samples. A strategy based on the consensus in the literature has been incorporated into the portfolio methods. These methods calculate the reasonable *N*-factor upper and lower limits for a given material. The limits are calculated using data from an amino acid (AA) analysis with multiple hydrolysis times and an accurate total nitrogen analysis substantially similar to the methods described by Mossé.[127]

Other Feedstock Constituents

Validated standard analytical methods are available for a variety of minor constituents or constituents specific to a small class of biomass substrates, examples being pectin, cutin, and phytate. Inclusion of these methods into the analysis portfolio increases mass closure and allows tracking of minor constituents that may be concentrated during the biomass conversion process.

Degradation Products

The liquid portion of biomass-derived process samples may also contain carbohydrate degradation products, such as 5-(hydroxymethyl)-2-furaldehyde (HMF), levulinic acid, and furfural, as well as other components of interest, such as organic acids and sugar alcohols. Portfolio methods are available for the quantitative measurement of these degradation products and byproducts of polymer hydrolysis.

Reconstructing the Composition of the Original Biomass Sample

Following the multistep sequence of the portfolio methods, the constituents' values can be reported in many forms. Because biomass polymers incorporate one molecule of water in the hydrolysis of each monomer molecule, the carbohydrate content of a feedstock may be reported as the theoretical yield of monomeric sugars for the assessment of the conversion efficiency of a biomass process. More commonly, constituent values in solid samples are reported as percent dry weight on either an extractives-free basis or as-received basis. The composition of liquid samples is reported as concentrations in grams per liter for each constituent. Three additional measurements are required to reconstruct the composition of biomass process slurries: total slurry weight, liquor density, and fraction insoluble solids (FIS). The total slurry weight minus the weight of insoluble solids provides the weight of the liquid portion of the sample. Dividing by the density measurement converts this to volume. With the volume measurement, constituent concentrations can be converted to recovered weight for the determination of constituent and process mass closure.

Rapid Analytical Methods. One approach to industrial biomass analysis is to use published,

standard methods to calibrate rapid, inexpensive spectroscopic techniques, which can then be used for feedstock and process analysis. Rapid analysis is a generic term for methods that couple traditional wet chemical methods of analysis with rapid, inexpensive spectroscopic techniques. The methods described here are often further classified as rapid biomass analysis methods. Although many spectroscopic techniques can be incorporated into rapid analysis methods, for biomass analysis, near infrared (NIR) spectroscopy has several advantages over alternate spectroscopic tools. Techniques have been developed for obtaining quality NIR spectra from bulk samples, minimizing the time and expense of sample preparation. Robust NIR instruments are commercially available for process analysis, process control, and field applications. Many of the industrial NIR techniques have been developed for use by the chemical, food processing, and agriculture industries and can be applied directly to the characterization of biomass.

Several steps are involved in rapid analysis method development. These include gathering appropriate calibration samples, chemical characterization of the calibration samples, developing spectroscopic methods for the rapid technique, projection-to-latent-structures (PLS) regression, validation of the PLS algorithm, and the development of QA/QC procedures.[128]

Rapid analysis methods based on PLS multivariate modeling require calibration based on robust and accurate methods. The first step in developing a new method is to gather appropriate calibration data. Robust methods usually contain at least 100 well-characterized samples. Collecting and characterizing a good calibration set cost about $300,000. This is by far the most expensive and time-consuming step in method development. Calibration samples should have compositions similar to the samples to be analyzed. If possible, the calibration set should include samples that represent all known sources of compositional variance. The range of compositional variability within the calibration samples determines the validated calibration range for each constituent.

Quality spectroscopy is the second essential component of method development. The technique selected must contain information about the chemical composition of each sample. The spectroscopic method is the key to cost reduction and speed of analysis. In the next step of rapid analysis method development, multivariate analysis is used to identify spectroscopic patterns that correlate with compositional data. In the methods incorporated into work at the National Renewable Energy Laboratory, projection-to-latent-structures models use whole NIR spectra collected from 400 nm to 2500 nm. Equations are obtained that convert spectroscopic data directly into compositional information. In simplified terms, PLS analysis solves hundreds of equations in thousands of variables to obtain a linear equation that predicts compositional information from spectroscopic data. Multivariate analysis is designed for complex systems such as those found in biomass compositional analysis. These powerful mathematical techniques retain the precision and accuracy of the calibration data. It is important to note that PLS analysis can never be more accurate than the methods used to obtain the calibration data.[97] For this reason, the best and most accurate wet chemical techniques should be used for method calibrations. Once calibration is complete, compositional analysis becomes as fast and inexpensive as the spectroscopic method.

One of the major limitations of rapid analysis methods is that an answer is always provided and the user must determine the validity of the provided data. Robust QA/QC procedures are needed to assure that the rapid methods are appropriately applied to unknown samples.[128]

In addition to significant savings in time and money for routine process samples, rapid analysis methods can be used to provide levels of information that were not previously available. For example, feedstock assessment and genetic studies require the screening of hundreds, sometimes thousands of samples. These studies would have been too costly to pursue without the savings in time and cost provided by rapid analysis methods. For example, approximately 200 samples can be analyzed from a bulk corn stover feedstock in one day at a cost of less than $4000. This type of information has been used to develop

protocols for the representative sampling of fields, bales, totes, and bags of feedstock. The ability to accurately sample a bulk feedstock and to analyze hundreds of samples for about $20 each provides a new tool that is being used to assess the compositional variability of corn stover in the United States as a function of variety, geographical location, harvest time, and collection method. Changes in feedstock composition during storage are also being monitored. With these larger data sets, feedstock composition can be more accurately reported as a range of expected normal values.

APPLICATION OF METHODS

Rapid, inexpensive biomass analysis can be useful at many stages of an industrial process. Rapid biomass analysis methods can characterize the feedstock as it enters the reactor. If necessary, rapid analysis can be used to guide feedstock blending. Chemical changes during the processing of biomass can provide feedforward and feedbackward information that can be used to ensure that the process maintains a steady state in spite of the feedstock variability. Finally, process residues and products can be easily evaluated to assess overall process economics. As more samples are analyzed, information can be obtained about the composition of an "ideal feedstock." Field-mobile instruments can be calibrated for use as purchasing tools. Buyers can obtain compositional information about a biomass feedstock at the point of purchase. Feedstocks can be assigned values based on quality. Young plants and perhaps even seeds can be evaluated and selected for desirable characteristics and production potential.

Biomass Process Monitoring and Improvement

The wet chemical and rapid analytical methods described above are being used on a daily basis in the U.S. Department of Energy's Office of the Biomass Program. The methods are used primarily to keep track of chemical fractions of plant cell walls during biomass processing. Researchers use this capability to better characterize biomass conversion processes and to compare and evaluate alternate approaches to biomass conversion. Methods have been developed that are specific to and appropriate for determining the composition of raw biomass materials (e.g., corn stover, softwoods, and hardwoods[129]), as well as processing intermediates (e.g., dilute-acid pretreated hardwoods, softwoods, and corn stover[128]). The high cost of compositional analysis using traditional wet chemical methods was limiting the amount of information available for economic assessments and process optimization. The additional levels of information provided by rapid analysis methods increase the accuracy of technoeconomic models that guide research more efficiently toward the development of commercially viable process options for the conversion of biomass into fuels and valuable chemicals.

Survey of Corn Stover Compositional Diversity

The corn stover feedstock rapid analysis method has also been used extensively to assess the range of compositional variation present in corn stover from different varieties grown in different locations using a variety of crop management and harvesting strategies. In one study, university agronomy department researchers, agricultural biotechnology and seed companies, and individual farmers all provided stover samples. This was an example of an application that would not and could not have been undertaken using traditional wet chemical methods, because they are both too slow and too expensive. The resource limitations disappeared to a great extent with the development of the rapid analytical methods. This survey has provided indispensable information regarding the range of diversity of corn stover and its impact on biomass conversion process economics.

Using rapid analysis, the National Renewable Energy Laboratory has begun compiling a corn stover composition database. The

database includes samples from 53 different locations in 10 states (mostly U.S. corn belt) and more than 100 branded hybrids from 22 seed companies. Individual samples typically represent about 10 to 20 stalks of the same variety collected from the same field and grown during the 2001 season. Stalks were collected by hand after grain harvest was completed, dried to less than 20 percent moisture at less than 50°C, and shipped to NREL. Stover samples in this survey do not include cobs. Samples were milled using a rotary knife mill (Wiley Mill) to pass a ¼-inch screen prior to NIR spectroscopy.

The NIR spectrometer used for method development and sample analysis was a Foss NIR Systems Model 6500 Forage Analyzer with a sample transport module and a standard reflectance detector array. The transport module moves the sample compartment up and down during data collection, thereby allowing a more representative spectrum to be obtained from bulky heterogeneous samples. The reflectance array uses two silicon detectors to monitor visible light from 400–850 nm and four lead-sulfide detectors to monitor NIR light from 850–2500 nm. Natural product sample compartment cells in 1/4-cup and 1-cup sizes were used as sample holders in the transport module. This instrument has a maximum resolution of 2 nm.

A key step in the application of rapid analysis methods is the collection of high quality NIR spectra. To minimize the effect of water in the biomass spectra, each sample was air-dried to less than 10 percent moisture prior to NIR spectroscopic analysis. Spectroscopic techniques were used that enable a high quality, reproducible, and representative NIR reflectance spectrum to be obtained. For each sample, a total of 35 spectra were collected and averaged to compensate for sample heterogeneity. Each calibration sample was subsampled three times. Sample cells were emptied and repacked between subsamples. Instrument reproducibility tests demonstrated that the reproducibility limits of the NIR spectrometer contributed less than 0.1 percent to the absolute prediction errors in the rapid analysis method.

NIR data were converted to compositions using the "stover 5c" rapid analytical method developed at NREL.[129] The ability of the stover 5C methods to accurately measure the composition of corn stover feedstock is shown in Fig. 33.17, where the composition, as determined by NIR/PLS, is compared to measurement of the same samples using standard wet chemical methods.

Improvements in the wet chemical calibration methods and the incorporation of additional calibration samples are continuously improving these rapid biomass analysis methods. In the future, information on these samples of corn stover can be updated using improved methods by reevaluation of their stored electronic spectra.

Figure 33.18 summarizes the results of compositional analysis by showing the distribution of values for total structural sugars (not including uronic acids) as a frequency histogram. Note both the normal distribution and the wide range of values. This same kind of normal distribution with a broad range is echoed by each of the individual major constituents of corn stover (i.e., glucan, xylan, lignin, protein, structural inorganics) across the population of 738 samples (data not shown). The dotted line in Fig. 33.18 indicates the composition of the corn stover used for process modeling efforts in the technoeconomic model by Aden et al.[130] The fact that the composition assumed for process modeling efforts now appears somewhat optimistic in light of these results has caused subsequent modeling efforts to reconsider the feedstock composition assumptions.

Compositional variability can have a significant impact on biomass conversion process economics. The large effect (i.e., at least $0.30/gal ethanol) of observed compositional diversity on process economics is shown in Fig. 33.19 and is primarily due to the fact that the maximum theoretical product yield is proportional to feedstock carbohydrate content (Fig. 33.20).[131] Yield is the major economic driver for the technoeconomic model used to assess the economic impact of composition on minimum product selling price,[130] as can be seen from the data in Fig. 33.21.

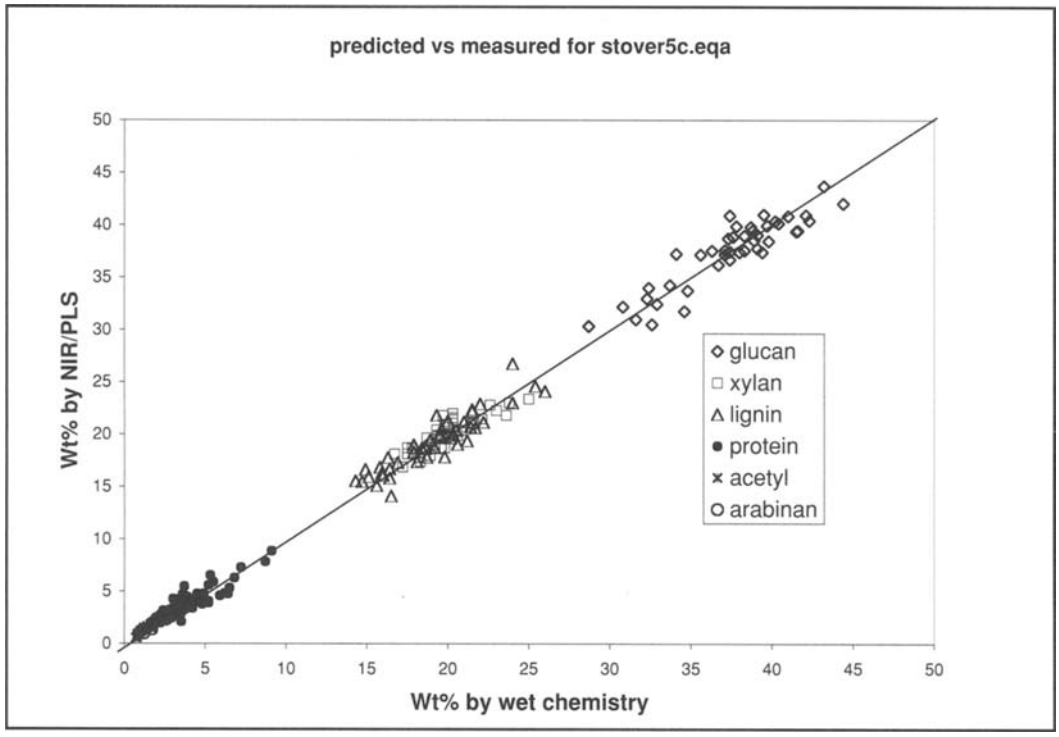


Fig. 33.17. Comparison of corns stover feedstock composition as determined by wet chemical and NIR/PLS method stover 5C.

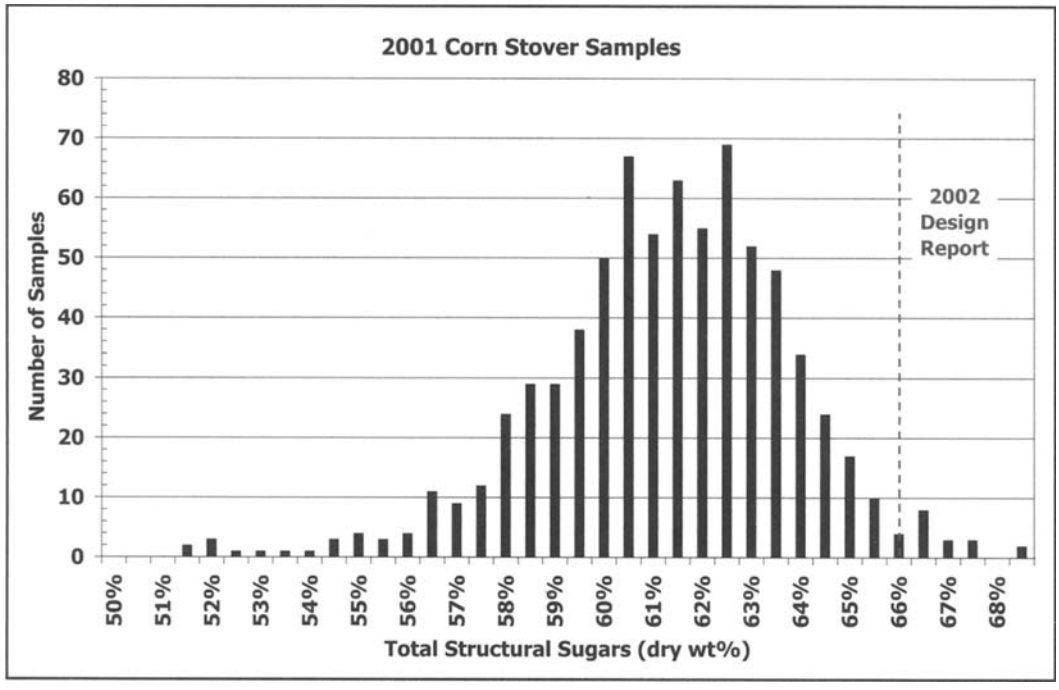


Fig. 33.18. Distribution of total structural sugars content among 738 hybrid corn stover samples collected in 2002.

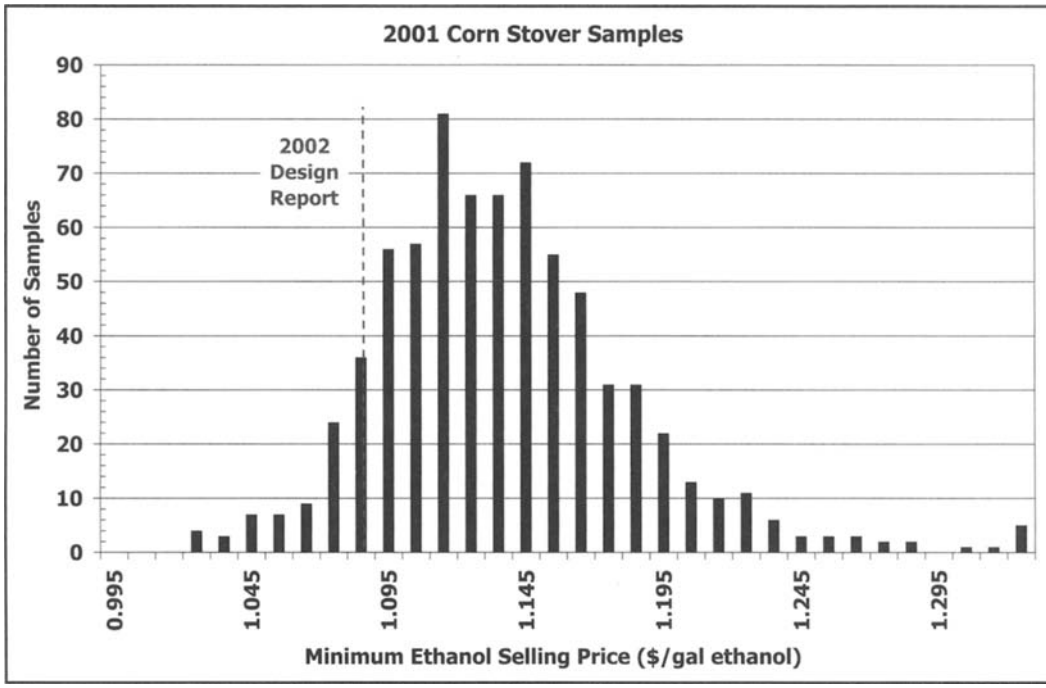


Fig. 33.19. Effect of feedstock composition on minimum ethanol selling price (MESP).

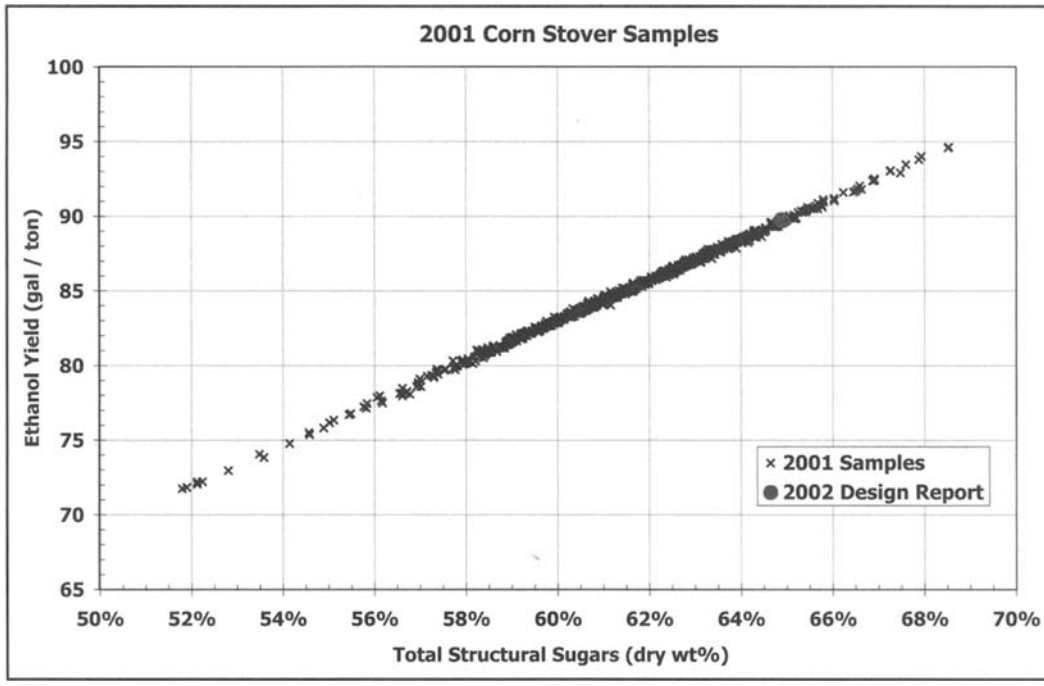


Fig. 33.20. Effect of feedstock composition on minimum ethanol selling price (MESP).

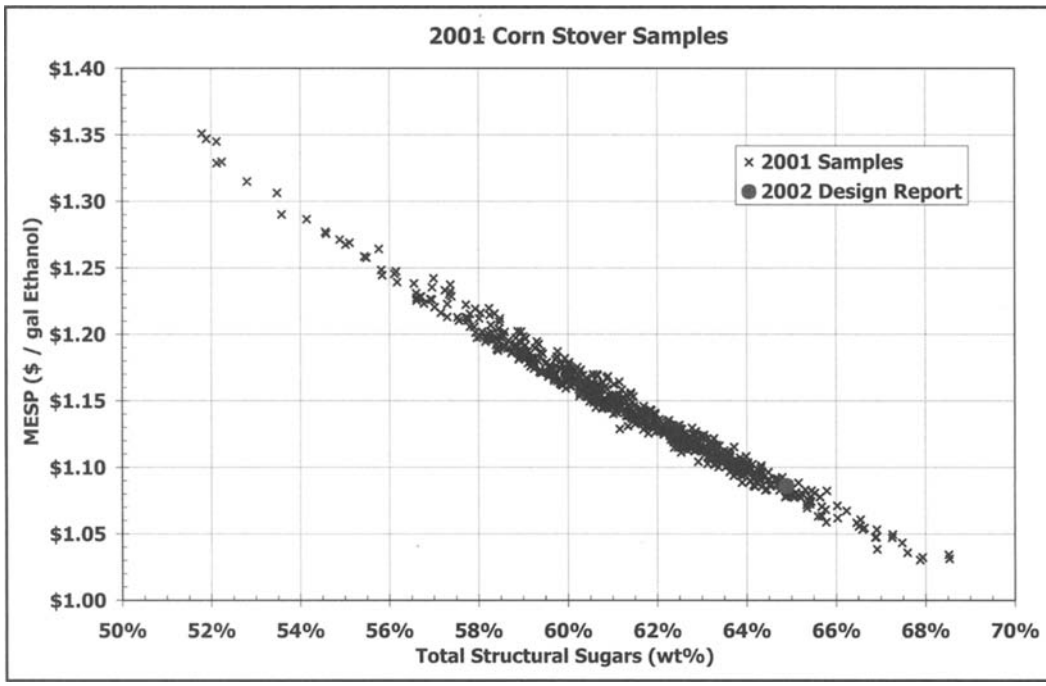


Fig. 33.21. Relationship between carbohydrate content of feedstock and ethanol yield per ton of input material.

Genetic Screening and Cell-Wall Genomics

The identification and characterization of genes involved in cell wall biogenesis can be approached using either a traditional genetic screening or a reverse genetics strategy. Both approaches are currently being used.[116–118]

A forward genetics approach requires the availability of a relatively inexpensive, high-throughput method to assess the phenotype of individuals in a population. In the case of visible phenotypes, this only requires a person to inspect a large number of plants to identify unusual individuals to capture the genetic variants of interest. In the case of chemical phenotypes this is somewhat more difficult, but certainly possible. Chemical screens have been performed previously to find mutations in genes that affect important metabolic pathways and result in accumulation of abnormal branching patterns in starch,[132] altered seed protein composition,[133] abnormal fatty acids accumulating in storage or membrane lipids,[134] or abnormal cell wall composition.[135,136]

Near-infrared spectroscopy has been employed as a high throughput screening tool to collect chemical information from plant tissue in a large genetic screen of a genomewide, transposon mutagenized collection of corn mutants.[116,137]

FUTURE APPLICATIONS

One of the long-term goals of rapid biomass analysis method development is the generation of robust methods for online and atline process monitoring and control. Because each method is feedstock, process, and sample specific, the achievement of this goal will require a significant long-term effort and considerable financial resources. One step in this direction is the development of automated and robotic methods of wet-chemical analysis that improve sample analysis throughput and decrease the cost of obtaining accurate calibration data.

The development of rapid biomass analysis methods is limited within the DOE program to feedstocks and processes currently used in

program demonstration projects and industrial partnerships. Methods for other potential feedstock materials such as wheat straw, switchgrass, sorghum, or alfalfa could be developed if sufficient need and resources are identified.

SUMMARY AND CONCLUSIONS

Accurate, reliable, precise, and inexpensive methods for determining the composition of biomass at various stages during a conversion process are essential to effectively monitor the progress of the thermochemical and biochemical processes. This information enables the comparison of various process options and enables intelligent decision making in evaluating process options for maximizing product yield while minimizing capital and operating costs. Eventually, with the development of feedback and feedforward loops, a commercial biomass conversion process can be monitored and controlled by a set of automated processes. The availability of more realistic information concerning sources of variability in commercial biomass conversion processes minimizes investment risk and increases the realistic probability of achieving success in the DOE goals in establishing commercial biomass conversion industries in the United States.

BIOCATALYSTS FOR BIOMASS DECONSTRUCTION

SUMMARY OF PLANT CELL WALL STRUCTURE

Plant cell walls are composed primarily of cellulose, hemicellulose, lignins, and pectins. These give structural rigidity and strength to the plant, deter pathogens, and retain extracellular water. Cellulose, a highly crystalline, insoluble polymer of β-(1,4)-cellobiose, comprises about 50 percent of the plant biomass. Although cellulose does not degrade easily, it will hydrolyze to glucose by the synergistic action of three distinct classes of enzymes: endoglucanases, exoglucanases, and cellobiases.[138,139] In contrast with the insoluble linear cellulose homopolymer, hemicelluloses are water- or base-soluble heteropolymers, comprised of a variety of branched and substituted polysaccharides. In addition to providing water retention and structural reinforcement, hemicelluloses act as crosslinking agents.

It is worth noting that although hemicelluloses are considered soluble in their native form in vivo, extraction can result in significant debranching, often rendering them insoluble in water. Recently, Gatenholm and Tenkanen assembled a collection on properties, uses, and examinations of various hemicelluloses, both in industrial and basic science applications.[140] The complex structure of hemicelluloses has dictated an accordingly diverse array of hemicellulases. Generally, each structural feature in hemicellulose has an associated enzyme that can hydrolyze or modify this feature. Pectins are acidic polysaccharides that retain large amounts of water and act as an adhesive between adjacent plant cells, and, along with lignin, comprise much of the middle lamella. Although pectin may be found to some extent in most plant cell walls, it is a minor component in most tissues (fruits being the most notable exception), and its degradation is not covered here. For reviews on biodegradation by and commercial uses of pectinases, see articles by Galante and Formantici, Hoondal et al., Kashyap et al., Lebeda et al., and Naidu and Panda.[141–145] Lignin, by contrast, is a heterogeneous polymer of phenyl propanoid units containing various phenolic derivatives. Lignin is often thought of as the binder that cements the cell wall components together. Enzymatic degradation of lignin is still somewhat controversial, with various hydrolytic and oxidative mechanisms proposed. Many papers have been published on white rot degradation of lignin in recent years, however, the authors are unaware of a unified complete mechanism of lignin degradation, and an overview is not attempted here. Several reviews have, however, covered selected areas including lignin degradation by mycorrhizal fungi, dye decolorization by white rot fungi, lignin biodegradation in compost, applications in pulp and paper and ruminant feed, and the emerging molecular genetics of ligninolytic fungi.[146–152]

ENZYMATIC HYDROLYSIS OF PLANT CELL WALL COMPONENTS

The complex nature and interconnectivity of plant cell wall polymers preclude straightforward enzymatic digestion. There are dozens of enzyme families involved in plant cell wall hydrolysis, including cellulases, hemicellulases, pectinases, and lignin-modifying enzymes. The Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB) has classified cellulases and hemicellulases, like all enzymes, into different classes based on activity. Table 33.2 and Table 33.3, compiled from the IUBMB enzyme nomenclature database (http://www.chem.qmul.ac.uk/iubmb/enzyme/), list the IUBMB enzyme classifications for cellulases and hemicellulases.[153]

Synergy is a major factor in degradation efficiency, making measurement of these activities difficult, to say the least. The biomass degrading enzyme system, encompassing cellulases, hemicellulases, pectinases, lignin-modifying enzymes, and other accessory activities, is far too complex to be covered in detail in this general overview. The American Chemical Society (ACS) has covered much of this work in the ACS Symposium series including applications to lignocellulosics, fiber processing, pulp and paper, biomass for fuels, biomass conversion, and general activities on insoluble carbohydrates.[154–160] Readers are encouraged to find details for specific applications in these proceedings. The burgeoning number of scientific informational databases accessible through the Internet has also helped greatly to clarify (to some extent) the confusing nature of these enzymes. Sites include general enzyme databases, such as ExPASy (http://us.expasy.org/, the server gateway to Swiss-Prot/TrEMBLE [http://us.expasy.org/sprot/] and ENZYME [http://us.expasy.org/enzyme/]) and BRENDA (http://www.brenda.uni-koeln.de), as well as databases devoted exclusively to carbohydrases, such as CAZy (http://afmb.cnrs-mrs.fr/CAZY/acc.html).[161–164]

Current literature describing the assaying of general cellulase activity (or of individual component enzymes) has broadened considerably since Mandels et al.[165] reported reducing sugar release and substrate weight loss as suitable cellulase assay methods. To some extent, and for appropriate substrates, these methods are still considered adequate. However, modern assays based on molecular weight (MW) analysis detected by high-performance liquid chromatography size-exclusion chromatography (HPLC-SEC), coupled enzymes, viscometry, hydrolysis of dyed or derivatized insoluble and soluble polymers, and hydrolysis of derivatized or labeled low-MW substrates, have greatly enhanced the understanding of these complex systems. Cellulose structure and physical disruption of cellulose microfibrils have also been

TABLE 33.2 Major Cellulase Enzyme Classes

IUBMB EC No. *Oligomer Hydrolase*	*Common Name*	*Activity*
EC 3.2.1.21	β-glucosidase	Hydrolysis of terminal, nonreducing β-D-glucose residues with release of β-D-glucose
Exo-Depolymerases		
EC 3.2.1.74	glucan 1,4-β-glucosidase	Hydrolysis of 1,4-linkages in 1,4-β-D-glucans, to remove successive glucose units
EC 3.2.1.91	cellulose 1,4-β-cellobiosidase	Hydrolysis of 1,4-β-D-glucosidic linkages in cellulose and cellotetraose, releasing cellobiose from the nonreducing ends of the chains
Endo-Depolymerase		
EC 3.2.1.4	cellulase	Endohydrolysis of 1,4-β-D-glucosidic linkages in cellulose, lichenin, and cereal β-D-glucans

TABLE 33.3 Enzyme Classes Involved in Hydrolysis of Hemicelluloses

IUBMB EC No.	*Common Name*	*Activity*
Debranching Enzymes		
EC 3.1.1.6	acetylesterase	An acetic ester + H_2O = an alcohol + acetate
EC 3.1.1.72	acetylxylan esterase	Deacetylation of xylans and xylo-oligosaccharides
EC 3.1.1.73	feruloyl esterase	feruloyl-polysaccharide + H_2O = ferulate + polysaccharide
EC 3.2.1.131	xylan α-1,2-glucuronosidase	Hydrolysis of α-D-1,2-(4-*O*-methyl)glucuronosyl links in the main chain of hardwood xylans
EC 3.2.1.139	α-glucuronidase	An α-D-glucuronoside + H_2O = an alcohol + D-glucuronate
EC 3.2.1.55	α-N-arabinofuranosidase	Hydrolysis of terminal non-reducing α-L-arabinofuranoside residues in α-L-arabinosides
Oligomer Hydrolases		
EC 3.2.1.22	α-galactosidase	Hydrolysis of terminal, nonreducing α-D-galactose residues in α-D-galactosides, including galactose oligosaccharides, galactomannans and galactohydrolase
EC 3.2.1.23	β–galactosidase	Hydrolysis of terminal nonreducing β-D-galactose residues in β-D-galactosides
EC 3.2.1.25	β–mannosidase	Hydrolysis of terminal, nonreducing β-D-mannose residues in β-D-mannosides
Exo-Depolymerases		
EC 3.2.1.100	mannan 1,4-mannobiosidase	Hydrolysis of 1,4-β-D-mannosidic linkages in 1,4-β-D-mannans, to remove successive mannobiose residues from non-reducing ends
EC 3.2.1.145	galactan 1,3-β–galactosidase	Hydrolysis of terminal, non-reducing β-D-galactose residues in (1,3)-β-D-galactopyranans
EC 3.2.1.37	xylan 1,4-β–xylosidase	Hydrolysis of 1,4-β-D-xylans, to remove successive D-xylose residues from the non-reducing termini
EC 3.2.1.58	glucan 1,3-β–glucosidase	Successive hydrolysis of β-D-glucose units from the nonreducing ends of 1,3-β-D-glucans, releasing α-glucose
EC 3.2.1.72	xylan 1,3-β-xylosidase	Hydrolysis of successive xylose residues from the nonreducing termini of 1,3-β-D-xylans
Endo-Depolymerases		
EC 3.2.1.101	mannan endo-1,6-α-mannosidase	Random hydrolysis of 1,6-α-D-mannosidic linkages in unbranched 1,6-mannans
EC 3.2.1.136	glucuronoarabinoxylan endo-1,4-β-xylanase	Endohydrolysis of 1,4-β-D-xylosyl links in some glucuronoarabinoxylans
EC 3.2.1.151	xyloglucan-specific endo-β-1,4-glucanase	xyloglucan + H_2O = xyloglucan oligosaccharides (endohydrolysis of 1,4-β-D-glucosidic linkages in xyloglucan)
EC 3.2.1.32	xylan endo-1,3-β-xylosidase	Random hydrolysis of 1,3-β-D-glycosidic linkages in 1,3-β-D-xylans
EC 3.2.1.39	glucan endo-1,3-β-D-glucosidase	Hydrolysis of 1,3-β-D-glucosidic linkages in 1,3-β-D-glucans
EC 3.2.1.6	endo-1,3(4)-β-glucanase	Endohydrolysis of 1,3- or 1,4-linkages in β-D-glucans when the glucose residue whose reducing group is involved in the linkage to be hydrolyzed is itself substituted at C-3
EC 3.2.1.71	glucan endo-1,2-β-glucosidase	Random hydrolysis of 1,2-glucosidic linkages in 1,2-β-D-glucans
EC 3.2.1.73	licheninase	Hydrolysis of 1,4-β-D-glucosidic linkages in β-D-glucans containing 1,3- and 1,4-bonds
EC 3.2.1.78	mannan endo-1,4-β-mannosidase	Random hydrolysis of 1,4-β-D-mannosidic linkages in mannans, galactomannans and glucomannans
EC 3.2.1.8	endo-1,4-β-xylanase	Endohydrolysis of 1,4-β-D-xylosidic linkages in xylans
EC 3.2.1.89	arabinogalactan endo-1,4-β-galactosidase	Endohydrolysis of 1,4-β-D-galactosidic linkages in arabinogalactans
EC 3.2.1.99	arabinan endo-1,5-α-L-arabinosidase	Endohydrolysis of 1,5-α-arabinofuranosidic linkages in 1,5-arabinans

examined using light, electron, and more recently, atomic force microscopy.[166–173] These types of "assay" are more useful when the properties of the cellulose fiber are in question, not when the goal is total hydrolysis. There has also been work using gel-permeation chromatography to characterize changes to cellulose structure by examining the products of cellulase action on wood fiber.[174] As with much of biotechnology today, high-throughput methods have also been developed to increase the speed and accuracy of cellulase assay.[175] Despite all of the assays, or perhaps because of them, workers in the field are reminded that only assays designed to measure the conversion of cellulose from the actual biomass substrates in question are ultimately valid performance measures.

Most enzymes have very specific requirements for tight substrate binding and precise transition-state formation, which usually leads to high catalytic turnover rates. However, even ideal catalytic sites must be "carried to the substrate" by the macromolecule within which it is housed. Enzymes are also large compared to the polysaccharide oligomers under attack, especially as the particular site of action may be buried in a heterocrystalline structure of mixed polysaccharides. The complexity of biomass, specifically in regard to hemicellulose structure, requires a high degree of coordination between degradative enzymes. Studies show correlations between the enzymatic digestibility of cellulose and the removal of hemicellulosic sugars and lignin, supporting the notion of close spatial relationships.[176,177] Of further complication is that the actions of glycosyl hydrolases often change the chemical environment of the partially degraded substrate, which in turn affects the actions of other glycosyl hydrolases. For example, partly because of the substituents attached to the main chain, most hemicelluloses are quite water soluble in their native state. These side chains disrupt the water structure and help to solubilize the hemicellulose. Debranching enzymes that remove these substituents generally decrease substrate solubility, and in turn lower the polysaccharide's susceptibility to endo-acting hydrolases.[178] Thus, a xylan that has been subjected to acetyl xylan esterase is less susceptible to enzymatic degradation than a xylan subjected to a mixture of branching and debranching enzymes.[179] As the substituents are removed, xylan can become less soluble, forming aggregates that sterically hinder and finally block further degradation.[180] The endoxylanases, for example, cleave the main chain linkages and are often quite particular about the type of linkage, type of sugar, and presence or absence of nearby substituents.[181] An endoxylanase that cleaves β-(1,4) linkages will usually have no effect on β-(1,3) linkages. Also, an endoxylanase that cleaves main-chain linkages near an *O*-2 linked arabinose may have no effect on an open-chain xylan.[182]

CELLULASES

Ask an industrial biochemist about cellulases and *Trichoderma reesei* will invariably come up. A mesophilic filamentous fungus typically found growing on cellulosic biomass, *T. reesei* produces arguably the most studied cellulase system in the world. Early research began when fungus-laden U.S. Army accoutrements were sent from the jungles of southeast Asia to the U.S. Army Natick Research and Development Laboratories during the 1940s.[183] This research program, led by Drs. Elwyn Reese and Mary Mandels for 40 years, succeeded not only in providing the U.S. Army and allies with chemical agents useful for protecting cotton goods, but also demonstrated the biological nature of their decay. The work at Natick demonstrated that many genera and species of fungi produce cellulose-degrading proteins, and that *T. reesei* was especially effective at degrading cotton. The Natick labs went on to learn how to grow *Trichoderma*, induce cellulase production, and determine operational ranges of pH and temperature, as well as tolerance to cations, anions, metals, solvents, and biomass-derived toxic compounds.[184] Furthermore, internship opportunities at Natick during the 1950s and early 1960s served to train the leaders of the next generation of cellulase scientists. In the end, this was perhaps one of the most important contributions of the program.

Despite cellulose's simple linear glucose polymer structure, its hydrolysis requires the coordinated action of at least three different types of β-1,4-glucanases (Table 33.2).

Endoglucanases

Endo-β-1,4-glucanases (EC 3.2.1.4) cleave the cellulose strand randomly along its length, generating new chain-end sugars, one reducing and one nonreducing. Usually, these chains remain insoluble and are thought to remain embedded in the cellulose crystal. The new ends, however, when exposed to the bulk water/cellulose interface, become new recognition sites for exocellulases.

Exoglucanases

Exo-β-1,4-glucanases (EC 3.2.1.91) cleave cellobiosyl (β-1,4-glucose dimers) or glucosyl units endwise from cellulose, chains. Most fungal, and some bacterial cellulase, systems display two types of exoglucanases: reducing-end and nonreducing-end cleavers. Exocellulases are thought to act processively on cellulose chains, starting at one end and proceeding down the chain. The products are either cellobiose or glucose, and along with the reducing/nonreducing end-specificity, are specific to the enzyme in question.

Endoglucanases and β-Glucosidases

When endo-activity occurs close to a chain end in an amorphous region of the cellulose, the new cellulose oligomer may become dissociated from the larger structure. The degree of polymerization of the chain determines its solubility, ranging from highly soluble glucose dimer cellobiose to nearly insoluble cellohexaose. New soluble oligomers are usually acted upon by a third class of cellulases, the β-glucosidases (EC 3.2.1.21). This class acts primarily, if not exclusively, on soluble cello-oligomers, with decreasing specific activity as chain length increases. As their preferred substrate is cellobiose, they have also been termed cellobiases.

All three of these general activities are required for efficient and complete hydrolysis of cellulose to glucose. A conceptualized view of each typical cellulase action is shown in Fig. 33.22.[185] The process is initiated by an endocellulase (in this case cel5A from *Acidothermus cellulolyticus*), which nicks the cellulose chain (Fig. 33.22A), exposing a new reducing and nonreducing chain end. Exocellulases (in this case the reducing-end specific cel7A from *T. reesei*) find these nicks (Fig. 33.22B) and feed the ends through their active sites, cleaving off products in a processive manner (Fig. 33.22C). If cellobiose is the product, as in the case of cel7A, then β-glucosidase hydrolyzes it to two glucose molecules (Fig. 33.22D).

TOTAL CELLULASE ACTIVITY MEASUREMENTS

Each cellulase class has had its share of assay development. Assays that specifically measure each class are difficult to establish, as cross-reactivity between classes is greater than zero. However, there are assays that can be used to determine the relative ratios of these activities.

When using cellulases to degrade lignocellulosic material, total activity is usually the primary interest. The complexity of lignocellulose substrates and the huge heterogeneity in cellulase systems, however, create a quagmire for the cellulase enzymologist asked to determine the "activity" units of a particular cellulase. There are numerous methods for assaying cellulase activity, but most fall into one of three categories: sugar release (measured as reducing sugar), chain length reduction (measured by viscometry, light scattering, or size exclusion chromatography), and artificial substrate hydrolysis (measured by dye, chromophore, or fluorophore release).

The IUPAC Filter Paper Assay

In 1987, after significant effort, an international committee of cellulase researchers and the International Union of Pure and Applied Chemists (IUPAC) produced a procedure seeking to standardize cellulase activity measurements. This procedure uses microcrystalline cellulose and the dinitrosalicylic (DNS) acid method of Miller[186] to measure reducing

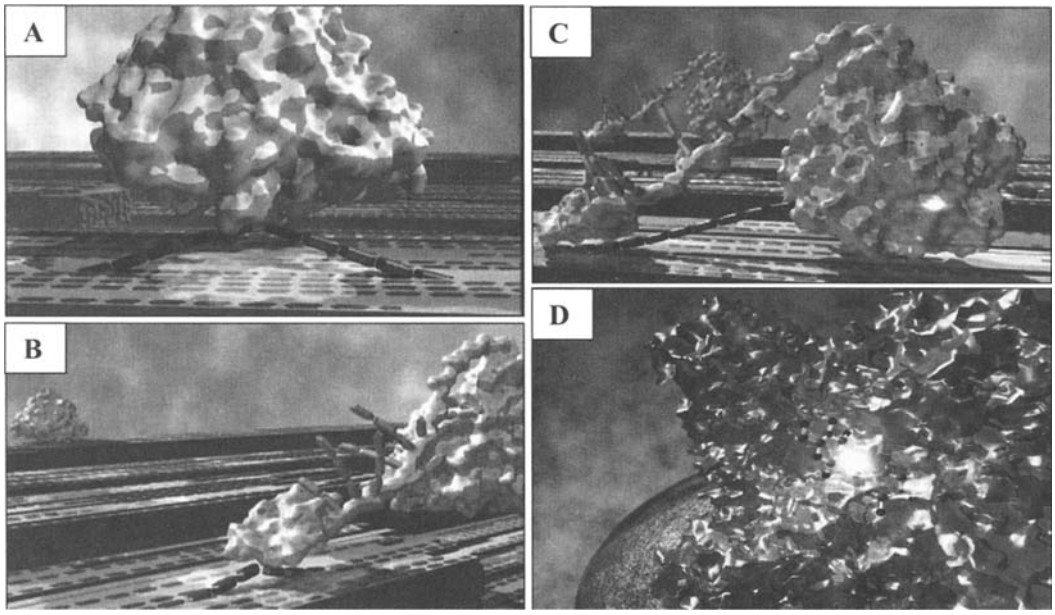


Fig. 33.22. Three enzyme activities in the degradation of cellulose: (A) endocellulase cel5A from *Acidothermus cellulolyticus;* (B) exocellulose cel7A from *Trichoderma reesei* finding an open reducing end; (C) cel7A processing along cellulose chain releasing cellobiose; and (D) bglA from *Bacillus polymyxa* hydrolyzing cellobiose.

sugars in the context of a highly specific assay protocol.[187] Because cellulose hydrolysis is nonlinear, the units were not defined as specific activity units per se (i.e., μmoles product/mg protein sec.), but rather as 4 percent hydrolysis of a 50-mg coupon of Whatman #1 filter paper, although many cellulase researchers have noted that after accounting for the addition of water to the glycosidic bond, the actual level of hydrolysis measured is 3.6 percent of the substrate. Regardless, the "filter paper unit" (FPU) was decreed the amount of enzyme that released 2.0 mg glucose from a 1.0×6.0 cm, 50-mg Whatman #1 test coupon after 60 min at 50°C. The dilution of enzyme required is converted, through a somewhat indirect procedure, to filter paper units (FPU) per mL. For example, an undiluted cellulase preparation that yields exactly 2.0 mg glucose has 0.37 FPU/mL. This fractional unit is the lowest cellulase activity measurable with the IUPAC assay.

The IUPAC cellulase assay has significant limitations and merely serves as the best-defined method. Extrapolation of required glucose release from highly diluted or concentrated enzyme solutions is not permitted; assays must be conducted with enzyme dilutions that closely bracket the actual value. Cellulase solutions too dilute to release 2.0 mg glucose must either be concentrated to an appropriate level, or determined incapable of assay by the IUPAC method. For highest accuracy, every working solution made from an enzyme stock must be reanalyzed for activity, a condition that complicates most analytical procedures.

Cellulase activities of *T. reesei* broths are normally reported to lie between 400 and 600 FPU/g total protein.[188] Our work to assess the specific activities of *T. reesei* cellulase preparations has led us to the direct comparison of commercial cellulase products, typically highly selected *T. reesei* mutants, and reconstituted, purified cellulase enzymes (Table 33.4). Although the range of specific activities found from this internally consistent study generally agrees with the literature, our estimation of the

TABLE 33.4 Specific Activities of Various *Trichoderma reesei* Cellulase Preparations

Source	*Mix of Component Enzymes*	*Specific Activity*[a] *(FPU/g protein)*	*Reference*
Multifect GC[b]	Native	431	Nieves et al.[681]
Ultra-low microbial cellulase 100[c]	Unknown	478	Nieves et al.[681]
Mutant strain RUT-C30	Probably nonnative	495	This study[d]
Spezyme lot #41-59034-004CL121	Native	565	Nieves et al.[681]
Cellulase TRL[e]	Native	569	Nieves et al.[681]
Spezyme lot #GC310 S1.2E2Z1P1	Native	571	Nieves et al.[681]
Mutant strain L27	Non-wild-type [682]	581	This study[d]
Mix CBH I:CBH II:EG I/60:20:10	Native mix [683]reconstituted	711	This study[f]
Mix CBH I:CBH II: EG I/40:50:10	Mix giving max activity	761	This study[f]

[a] Using the method of Ghose[187] (in order to facilitate comparison, all assays reported here were performed in a consistent manner).
[b] Spezyme and Multifect from Genencor International, South San Francisco, CA.
[c] Iogen Corporation, Ottawa, Ontario.
[d] Grown on 1% Sigmacel 50 + 2% lactose in Mandels media.[684]
[e] Cellulase TRL form Solvay Enzymes, Elkhart, IN.
[f] Reconsitituted mixtures from purified cellulase enzymes.

maximal specific activity of reconstituted *T. reesei* cellulases is somewhat higher, 760 FPU/g protein.

In fact, the activity of the native mixture of *T. reesei* cellulases [i.e., 10:20:60 for endoglucanase:cellobiohydrolase II:cellobiohydolase I (EG I:CBH II:CBH I)] using reconstituted enzymes is about 10 percent less than that obtained with a mix higher in CBH II (Table 33.4). One should be aware that it is possible to find reports in the literature of *T. reesei* cellulases with specific activities greater than about 600 FPU/g protein. Considering these findings in view of recent studies of the magnitudes of possible inter- and intralaboratory errors in filter paper[189] and protein assays,[190] assay errors or inconsistencies are most likely responsible for most discrepancies in specific activity reported.[191]

Variations on the filter paper assay have been developed. Irwin and coworkers have established a method for differentially measuring soluble and insoluble reducing sugars released from filter paper.[192] Ghose and the IUPAC committee also proposed a similar approach for measuring hemicellulases in 1987.[193] This method relies on meeting a standard level of conversion of the xylan fraction in oat spelt xylan to xylose in a specified period of time under standard conditions. This latter method; however, is not often cited in the literature.

General Non-IUPAC Cellulases Assays

Many cellulase enzyme preparations are simply not concentrated enough to cause the required release of 2 mg glucose from the 50-mg filter paper sample in 60 min. If these samples cannot be concentrated accurately (which is often the case), traditional FPU cannot be measured. In such cases, however, the IUPAC committee recommends that the reducing sugar release per unit time be accepted as a "provisional" measure of enzyme activity. This is similar to the pseudo-initial rate approach often used in the decade previous to the IUPAC report to measure cellulase activity from a wide variety of substrates. These substrates may include filter paper,[194] Avicel,[195] de-waxed cotton,[196] or phosphoric-acid-swollen cellulose (PSC).[197] Methods based on the use of antibiotic disks[198] and turbidity development[199] also predated the IUPAC study. Because all three cellulase types generate new reducing ends, the most direct measurement of cellulase activity is the detection of

new reducing ends in the cellulose chain. These methods are traditionally based on initial rate measurements introduced by Wood and McCrae in 1977.[195]

The reducing sugars are typically measured by the Somogyi[200] and Nelson[201] procedures, which measure reduction of Cu^{++} to Cu^{+} in alkaline solution, or by the dinitrosalisylic acid (DNS) assay, in which reducing sugars reduce DNS to 3-amino, 5-nitrosalicylic acid under alkaline conditions. Disodium 2,2'-bicinchoninate (BCA) can also be used to measure reducing sugars.[202] This reagent was found to be the best choice in a recent comparison of methods for the determination of endoglucanase activity.[203] The BCA method was highly sensitive, simple to perform, and directly gave the number of bonds broken, thus allowing for expression of endoglucanase activity in international units (μmol of β-1,4-glucosidic bonds hydrolyzed in 1 min during the initial period of hydrolysis).

Other methods used to measure cellulase activity include an automated filter paper assay for high throughput evaluation of cellulases,[175] a diafiltration saccharification assay for minimization of product inhibition and high degree of digestion,[204] measurement of cellulose size reduction by multiangle laser light scattering,[203] use of dye release from various dyed substrates, and differential staining of cellulose digestion products.[205–208]

Mathematical Modeling

A mathematical model has also been proposed for evaluating cellulase preparations. Sattler et al.[209] describe a relationship between hydrolysis extent, reaction time, and enzyme concentration. This procedure permits the effectiveness of different enzymes and of different pretreatment methods to be ranked. This method examines cellulose hydrolysis data collected from hyperbolic functions of substrate concentration versus cellulase enzyme concentration at various timed incubations. The model is based on a double reciprocal plot of the relationship

$$(Y/C_o)^{-1} = (K\,C_o/Y_{max})[E]^{-1} + (Y_{max}/C_o)^{-1}.$$

Y/C_o is the fraction of substrate hydrolyzed, $[E]$ is given in FPU/g substrate initially added, and Y_{max}/C_o is the fraction of substrate that could be maximally hydrolyzed at an infinite enzyme concentration. The y-axis intercept in the double reciprocal plot, $(Y_{max}/C_o)^{-1}$, may be used to quantify the quality of the enzyme preparation. Ideally, an enzyme should have a high Y_{max} and a low value for KC_o/Y_{max}. Adney and coworkers used this general method successfully to model the action of commercial *T. reesei* cellulase preparations on Sigmacell-50.[210] Results from double reciprocal plots of enzyme activity, (percent conversion)$^{-1}$, versus loading, (FPU/g cellulose)$^{-1}$, enabled extrapolation to infinite enzyme loading or maximal digestibility.

Endocellulase Activity Measurement

The "endo-1,4-β-glucanases" or 1,4-β-D-glucan 4-glucanohydrolases (EC 3.2.1.4), which act randomly on soluble and insoluble 1,4-β-glucan substrates, are commonly measured by detecting the decrease in viscosity or increase in reducing groups using carboxymethylcellulose (CMC).[211–213] It is thought that the methyl groups limit activity of the exoglucanases while still permitting the endo-types to hydrolyze the chain interior, perhaps in regions with low side-chain density. Endoglucanases are also capable of hydrolyzing various polymeric, substituted substrates, such as Ostazin brilliant-red hydroxyethylcellulose (OBR-HEC) and azo-dyed and cross-linked HEC (AZCL-HEC), as well as the low-MW fluorogenic substrates, such as 4-methylumbelliferyl-cellobiose (MUC), 4-methylumbelliferyl-lactopyranoside (MUL), or 4-methylumbelliferyl-cellotriose (MU-G3). Although these substrates make it easy to measure activity, care should be taken in relating the measured activities to performance on biomass, as the two substrates are not equivalent.

Viscometric Assays

Viscometric approaches to cellulase measurement activities are important because other methods measure only the number of glycosidic bonds cleaved in a polymeric substrate.

They do not provide any information about location in the substrate of the bonds cleaved. Viscometric methods measure a substantial change in a physical property of the substrate polymer, a very sensitive function of both the number and the location of the bonds cleaved.[214–216] For this reason, even though the recommended international units of carboxymethylcellulase are given in terms of glycosidic bonds cleaved, the most careful workers measure both bond cleavage (most often by measurement of sugar-reducing groups) and the change in solution viscosity as enzymatic hydrolysis proceeds. Vlasenko and coworkers[203] found the viscometric method to be simple to perform and highly sensitive for the internal bonds cleaved. However, this method does not account for the hydrolysis of CMC near the chain end and thus only allows for expression of endoglucanase activity in arbitrary viscometric units.

Exocellulase Activity Measurements

The "exo-1,4-β-D-glucanases" include both the 1,4-β-D-glucan glucohydrolases (EC 3.2.1.74), which liberate D-glucose from 1,4-β-D-glucans and hydrolyze D-cellobiose slowly, and 1,4-β-D-glucan cellobiohydrolase (EC 3.2.1.91), which liberates D-cellobiose from 1,4-β-glucans. Differentiation of these enzyme classes requires analytical techniques to distinguish glucose and cellobiose and is usually carried out by high-performance liquid chromatography (HPLC) or gas chromatography (GC). These enzymes can be further distinguished by their ability to liberate free sugars from either the reducing or nonreducing end of the cellulose chain.[217,218] Synergy studies with enzymes of known orientation are usually used to determine which preference a given enzyme has.[192,219,220]

The process of detecting and verifying exoglucanases has long been controversial. If purified proteins are available, careful comparisons of reducing sugar yields and fluidity values from CMC hydrolysis as a function of enzyme concentration can be used to judge whether an enzyme is more EG-like or CBH-like. Of course, purified enzymes can also be further verified by analyzing the product from hydrolysis of a series of derivatized (i.e., radiolabeled, chromophoric, or fluorophoric) cello-oligomers.[221,222] One class of these derivatives, cellobiosyl fluorides, has been reported to distinguish between CBH I and CBH II from *T. reesei* based on cleavage activity on the alpha and beta conformations of the cellobiosyl fluorides.[223] Claeyssens et al. also reported this type of rigorous analysis for fungal CBH I and CBH II.[224] Further specificities can be determined for proposed exoglucanases from analytical product evaluation by HPLC.[225,226] This is a much more definitive method of distinguishing endo- from exo-acting cellulases.

In general, exoglucanases such as CBH I can be expected to hydrolyze the aryl-substrates MUC and MUL at the agluconic bond, but not the substituted soluble celluloses such as AZCL-HEC, OBR-HEC, and CMC.[227] Phosphoric acid swollen cellulose is also used as a substrate for exoglucanases; however, some endoglucanases will hydrolyze this cellulose form as well.[226] Analysis of activity on higher oligomeric derivatives has proven to be challenging, especially with EGs and CBH I.[221] Furthermore, because endoglucanases are highly synergistic with exo-acting glucanases, the presence of endoglucanases significantly complicates efforts to quantify exoglucanase activity and can be compensated for only by the separate purification and kinetic characterization of the endoglucanase. Attempts persist to link this synergy effect to the cellulose-binding module (CBM). There is some evidence that the CBM alone can have a synergistic effect on the activity of fungal cellulases, both exo- and endo-acting types.[228–230]

β-Glucosidase Activity Measurements

The "β-D-glucosidases" or β-D-glucoside glucohydrolases (EC 3.2.1.21) act to release D-glucose units from cellobiose, soluble cellodextrins, and an array of glycosides. For cellobiose or cello-oligomers, this activity is usually measured and analyzed by HPLC or GC, or by direct spectrophotometric or fluorometric analysis of various chromogenic and

fluorogenic analogues of cellobiose and cello-oligomers. β-D-glucosidase/cellobiase activities are usually determined according to the method of Wood[231] as aryl-β-glucosidase activity by the hydrolysis of *p*-nitrophenyl-β-D-glucopyranoside. The concentration of *p*-nitrophenol is determined from the absorbance at 410 nm under alkaline conditions induced by the addition of 2 M Na_2CO_3. One unit of activity is defined as the amount of enzyme that catalyzes the cleavage of 1.0 μmol substrate per minute at 99°F (37°C). If necessary, β-D-glucosidases can be distinguished from other cellulases by the relative differences in the initial rates for aryl-β-D-glucosides and cellobiose. Also, the unique and acute sensitivity of β-D-glucosidase to inhibition by gluconolactone provides a method to assess exoglucanase activity in mixed systems of these two enzymes. This approach is necessary because β-D-glucosidase cleaves the agluconic, as well as the holosidic bond of aryl-glucosides.[232] Note that a similar approach is often used to assay other aryl-glycosidases.[233–235] This practice is made possible by the availability of many *o*- and *p*-linked aryl-glycosides including (but not limited to) β-xylosides, β-mannosides, β-galactosides, and L-arabinofuranosides.

HEMICELLULASES

In contrast to cellulases, the hemicellulases encompass a much broader suite of activities. In addition to analogue versions of endo-, exo-, and glycosidase cellulase activities, multiple debranching activities are needed to handle the high complexity of the heterogeneous hemicelluloses (Table 33.3). The varied backbone composition of hemicelluloses also adds complexity. Xylans, xyloglucans, mannans, and numerous other minor polysaccharide chains form the backbone for different hemicelluloses.

1. The endo-enzymes attack polysaccharide chains internally, with decreasing activity as chain length rises. Endoxylanases (EC 3.2.1.8) are specific for β-(1 → 4)-xylopyranose polymers, (i.e., the backbone of xylan) and others are specific for other hemicellulose polymers, such as mannan (endo-(1 → 4)-β-mannosidases, EC 3.2.1.78) or β-glucanases (endo-(1 → 3)-β-D-glucosidase, EC 3.2.1.39). As with endocellulases, these activities can be measured by viscometry or production of reducing sugar end groups on the appropriate hemicellulosic polymer.
2. The exo-enzymes act processively from either the reducing or nonreducing termini and again are specific to the type and length of the polymer. Some exo-acting enzymes have preferences for short chain substrates (DP 2-4), acting as cellobiase analogues, whereas others prefer larger substrates (DP > 4). Xylan (1 → 4)-β-xylosidase (EC 3.2.1.37), glucan (1 → 3)-β-glucosidase (EC 3.2.1.58), and mannan (1 → 4)-mannobiosidase (EC 3.2.1.100) are exo-acting enzymes specific for xylan, β-(1 → 3)-glucan, and mannan, respectively.
3. So-called "accessory" enzymes are also required for hydrolysis of hemicellulose in native plant tissue. These enzymes include a variety of acetyl xylan esterases (EC 3.1.1.72), acetyl esterases (EC 3.1.1.6), and esterases, such as feruloyl acid esterase (EC 3.1.1.73),[178] that hydrolyze lignin glycoside bonds. They also include classes for cleavage of specific hemicellulose side chains, such as α-L-arabinofuranose, glucuronic acid, and 4-*O*-methyl-glucuronic acid groups.

General Hemicellulase Assays

Xylan is the most predominant hemicellulose in most plant cell walls, comprising about ¼ to ⅓ of total plant biomass.[236] Xylans function primarily by forming crosslinks between the other cell wall components, such as cellulose, lignin, other hemicelluloses, and pectin. The term "xylan" is a catch-all for polysaccharides that have β-(1 → 4)-D-xylopyranose backbones with a variety of side chains usually attached at the O-2 and O-3 positions, and include glucuronic acid, 4-O-methylglucuronic acid, L-arabinofuranose,

xylose, and acetyl groups. The types and levels of side chains are dependent on the particular plant, with hardwoods having high acetyl and glucuronic acid moieties (glucuronoxylans) and grasses having mainly arabinofuranose and acetyl groups (arabinoxylan). Because of the complex nature of xylans, their enzymatic hydrolysis is intrinsically more complicated than that of most other plant polysaccharides. Typically, the polymer is debranched, either prior to, or in conjunction with, depolymerization of the backbone. Different debranching enzymes are required depending on the specific type of xylan being hydrolyzed. These include arabinofuranosidases, ferulic and coumaric acid esterases, acetyl and acetyl xylan esterases, glucuronidases, and xylosidases. Removal of these side chains synergistically enhances the rate of degradation by endoxylanase enzymes.[178,237–243]

Hemicellulose Debranching Enzymes

Debranching of the xylan backbone produces a wide variety of soluble low-molecular-weight compounds. Typically, these products are measured either by HPLC or GC. The difficulty in assaying these products is not so much in the detection as in obtaining the correct substrate for the enzyme. Most commercial xylan products are extracted by alkaline treatment, essentially hydrolyzing any ester linkages by saponification; that is, any acetyl-, coumaroyl-, or feruloyl esters are destroyed. Glycosidic side chains, such as arabinose or glucuronic acid are left intact, however, the polymer is typically insoluble. Enzyme studies using these substrates must be interpreted with caution, as the native esterified xylan is soluble. Extraction by DMSO or steam has been used to prepare "native" xylan, in which the esters are still intact and the polymer is soluble in water.[244]

Arabinofuranosidases. α-L-arabinofuranosidases (EC 3.2.1.55) cleave α-L-arabinofuranosides from the arabinoxylan xylose backbone and have been shown to enhance the release of ferulic and coumaric acid from arabinoxylan, presumably through a preference for hydrolyzing phenolic acid substituted arabinose side chains.[245] In the context of hemicellulose hydrolysis, the activity most often reported is hydrolysis of the α-(1 $\rightarrow$ 2)-glycosidic linkage of the arabinofuranoside to the xylan backbone. Some of these enzymes have been shown, however, to cleave linear or branched α-(1 $\rightarrow$ 5)-linked arabinan side chains found in some pectins, resulting in some confusion regarding the specificity of this enzyme class.[246–249] Although most assays are carried out on extracted arabinoxylan, p-nitrophenyl-arabinofuranoside has also been used as a substrate.[250–252]

Esterases. Acetyl esterase (EC 3.1.1.6) removes acetyl esters from acetylated xylose and short-chain xylo-oligomers. It's polymer-acting counterpart, acetyl xylan esterase (EC 3.1.1.72), has a similar activity, but prefers polymeric xylan.[244] In addition to acetate-specific enzyme detection kits, HPLC or GC analysis of acetate release from native extracted xylan and chemically acetylated xylan, colorimetric substrates, such as *p*-nitrophenol acetate and β-napthyl acetate, or the fluorometric substrate, 4-methylumbelliferyl acetate are also used to assay acetyl esterases.[244,253] The third esterase, ferulic acid esterase (EC 3.1.1.73), hydrolyzes the ester bond between ferulic acid or coumaric acid and the arabinose side chain of arabinoxylan. Assays for this activity are usually carried out using starch-free wheat bran or cellulase-treated gramineous biomass as a substrate and monitoring ferulic or coumaric acid released by HPLC or TLC. When preparing enzyme-treated substrates, care must be taken to employ phenolic-acid-esterase-free cellulases.[244] Other substrates include methyl and ethyl esters of the phenolic acids, as well as finely ground plant biomass.[240,254,255]

Glucuronidases. In hardwood xylans, xylan α-1,2-glucuronosidase (EC 3.2.1.131) and α-glucuronidase (EC 3.2.1.139) are involved in debranching the xylan backbone through removal of α-(1 $\rightarrow$ 2)-linked glucurono- and 4-*O*-methyl-glucuronosides.[256–259] Although relatively little work on these enzymes has been carried out, Tenkanen and Siika-aho

reported synergy with endoxylanase utilizing de-acetylated birch glucuronoxylan. The same report also demonstrated that acetylation interferes with glucuronidase activity and that higher activity was observed on soluble softwood 4-*O*-methylglucuronoxylan.[257] Such synergy has also been reported by others.[257,260,261] Para-nitrophenyl-α-D-glucuronide is used as a substrate for α-glucuronidase,[262] whereas xylan α-1,2-glucuronosidase is specific for an α-(1 $\rightarrow$ 2)-linked glucuronoside. Some glucuronidases, including membrane-bound enzymes, have been found to prefer glucuronoxylo-oligomers as substrates.[261,263–265] One report demonstrated the specific requirement for the 4-*O*-methyl group for efficient binding and positioning of the side chain in the enzyme active site.[258]

Hemicellulose Depolymerization Enzymes

As noted for cellulases, hemicellulose depolymerizing enzymes are divided into three classes; endo-acting enzymes, exo-acting enzymes, and oligomer-hydrolyzing enzymes. Although mechanisms of hemicellulose hydrolysis have been steadily studied over the years, they have not received the attention given to cellulose hydrolysis. Despite this, a general pattern of degradation is beginning to emerge. Although there are specific examples of endo-acting enzymes requiring side chains for maximal activity,[266] the majority of the endo-acting hemicellulose hydrolases tend to be more active on debranched or partially debranched hemicellulose, especially in the case of xylanases. The limitation on this increased activity is probably due to solubility or the polysaccharides, which tend to become more insoluble as the debranching process continues. Decreasing chain length from the activity of endo-hemicellulases mollifies this, allowing the shorter, less substituted fragments to remain soluble. Exo-acting enzymes, which probably fall into reducing- and nonreducing-end-specific groups and oligomer-hydrolyzing enzymes, also require debranching as a precursor to maximal activity. Overall, a balance must be met among removing the branching side chains from the polysaccharide backbone, decreasing the average chain length, and hydrolyzing the oligomers into free monomers, all while maintaining enough solubility of the fragments to allow enzyme access. The concerted action of the various hemicellulase enzyme classes probably accounts for the high synergy observed when the enzymes are used in concert.[260]

Xylanases. Depolymerization of the xylan backbone is mediated by endoxylanases with oligomers hydrolyzed by β-xylosidases. Structurally, the endoxylanases are divided into glycosyl hydrolase families 10 and 11. These enzyme families are similar in that they both depolymerize xylan via the Koshland-type, two-step catalysis that leaves products with retained stereochemistry of anomeric configuration. Family-10 enzymes typically yield lower molecular weight products (tetramers) than do family-11 enzymes (pentamers).[267] This is likely a function of the difference in binding sites, with family-10 enzymes having a binding site that recognizes shorter oligosaccharides than do family-11 enzymes.[268,269] Although these enzymes are active on native branched xylan, debranching may increase their activity.[261,270]

Assays for endoxylanases follow the same general patterns as endocellulase assays. Viscosity reduction, reducing sugar production, dye-release, solublization, zymogram analysis, and colorimetric/fluorometric analogues are all used to determine endoxylanase activity.[271–280] DNS detection of reducing sugars from xylan is the most cited method. Endoxylanases tend to have a preference for polymers of a certain degree of polymerization (DP). Bray and Clarke reported a *Schizophyllum commune* endoxylanase with a preferred substrate DP of seven,[281] whereas others reported enzymes exhibiting true endo-type activity, with decreasing activity at lower DPs.[266,282,283]

There are numerous reports of β-xylosidases that cleave short chain xylo-oligomers to xylose. In these cases, product detection was typically carried out by direct HPLC analysis or hydrolysis of p-nitrophenyl-β-D-xylopyranoside.[284–287]

Xyloglucanases. Xyloglucans are polysaccharide polymers composed of a linear backbone of β-(1 $\rightarrow$ 4) linked glucopyranose moieties with some monomers substituted with xylopyranose in an α-(1 $\rightarrow$ 6) linkage. The xylose side chains can in turn be substituted with one or more of the disaccharides α-(1 $\rightarrow$ 2)-L-fucosylpyranose-β-(1 $\rightarrow$ 2)-D-galactopyranose and α-(1 $\rightarrow$ 2)-L-galactopyranose-β-(1 $\rightarrow$ 2)-D-galactopyranose, with the fucose residues being found mainly in primary cell walls.[288–293] α–(1 $\rightarrow$ 2)-L-arabinofuranose has also been shown to be substituted onto either the main glucose chain or onto the xylose side groups.[288,294] It has been shown that xyloglucans are acetylated through *O*-linkages to the arabinose or galactosyl side chains.[288,292,294,295] Despite this side chain substitution, a specific acetylxyloglucan esterase has not been discovered.

There is an exo-acting enzyme that acts on the nonreducing end of xyloglucan oligomers. Oligoxyloglucan beta-glycosidase (EC 3.2.1.120) is produced by *Aspergillus oryzae* and removes an α-xylo-β-(1 $\rightarrow$ 6)-D-glucosyl dimer (isoprimverose) from the nonreducing end.

β-Glucanases. β-Glucan is a glucopyranose polymer containing either β-(1 $\rightarrow$ 3) or mixed β–(1 $\rightarrow$ 3), β–(1 $\rightarrow$ 4) linkages. The ratio of (1 $\rightarrow$ 4) to (1 $\rightarrow$ 3) linkages varies by species and gives specific properties to individual β-glucan polymers. Because of the differences in the linkages, different enzymes are required to cleave the two forms of β-glucan.[296–301] We now know that β-glucan can be degraded by glycosyl hydrolase family-12 enzymes (EC 3.2.1.4). Although these endo-acting enzymes are active on β-(1 $\rightarrow$ 4) glycosidic linkages, they are differentiated from other β-(1 $\rightarrow$ 4)-acting enzymes by the distinction of being able to hydrolyze the β-(1 $\rightarrow$ 4) linkages in mixed β-(1 $\rightarrow$ 3, 1 $\rightarrow$ 4)-linked polysaccharides. Glucan endo-1,3-beta-D-glucosidase (β-(1 $\rightarrow$ 3) glucanase) (EC 3.2.1.39) is an endo-acting glycosyl hydrolase that acts on β-(1 $\rightarrow$ 3) glucan, but has very limited activity on the mixed linkage β-glucan. Endo-1,3(4)-beta-glucanase) β-(1 $\rightarrow$ 3, 1 $\rightarrow$ 4) glucanase) (EC 3.2.1.6), is also an endo-acting glycosyl hydrolase. There is an exo-acting glycosyl hydrolase that is active on β-(1 $\rightarrow$ 3) glucan. Glucan 1,3-beta-glucosidase (EC 3.2.1.58) acts by processively releasing glucose from β-(1 $\rightarrow$ 3) glucan from the nonreducing end. One interesting report utilized an enzyme-linked sorbant assay in microtiter plates coated with biotinylated β-glucan to determine activity.[302]

Mannanases, Gluco- and Galactomannanases. Whereas mannan is characteristically described as a linear β-(1 $\rightarrow$ 4) mannopyranose polymer, galactomannan is composed of a polymeric β-(1 $\rightarrow$ 4) mannopyranosyl backbone highly substituted with β-(1 $\rightarrow$ 6) linked galactopyranose residues.[303,304] The degree of substitution varies with source. Glucomannan, found mainly in the root of the konjac plant (*Amorphophallous konjac*) consists of a β-(1 $\rightarrow$ 4) linked mannopyranose and glucopyranose backbone in a ratio of 1.6:1.[305] The backbone residues are substituted in a β-(1 $\rightarrow$ 3) linkage with several sugars and short oligosaccharides, as well as with *O*–linked acetyl groups about every 15 residues.[306–308] The structure, and hence the degradation, of mannan is very analogous to cellulose, both being linear β-(1 $\rightarrow$ 4) linked monosaccharide polymers.[309] Mannan, however, is found in only a few particular plants, notably in the endosperm of the ivory-nut from the Tagua palm (*Phytelephas macrocarpa*) and a few other plants.[309] In glucomannan and galactomannan, as with xylan, degradation requires both debranching and depolymerizing enzymes, which work in synergy.[242] There are two major types of galactomannan, differentiated mainly by the source and degrees of galactose substitution. locust bean gum, derived from the carob tree (*Ceratonia siliqua*), contains an average of 2000 sugar residues, with a galactose about every 3.5-mannosyl residues. The other major commercial source is guar gum, from the seed of the leguminous shrub *Cyamopsis tetragonoloba.* Guar gum contains more galactose residues than locust bean gum, having a galactose every 1.5 to 2 mannose units, and is longer, with residue counts of around 10,000.

The enzymes involved in depolymerization of the mannans consist of β-mannanase (EC 3.2.1.78), the endo-acting enzyme, and β-mannosidase (EC 3.2.1.25), which produces

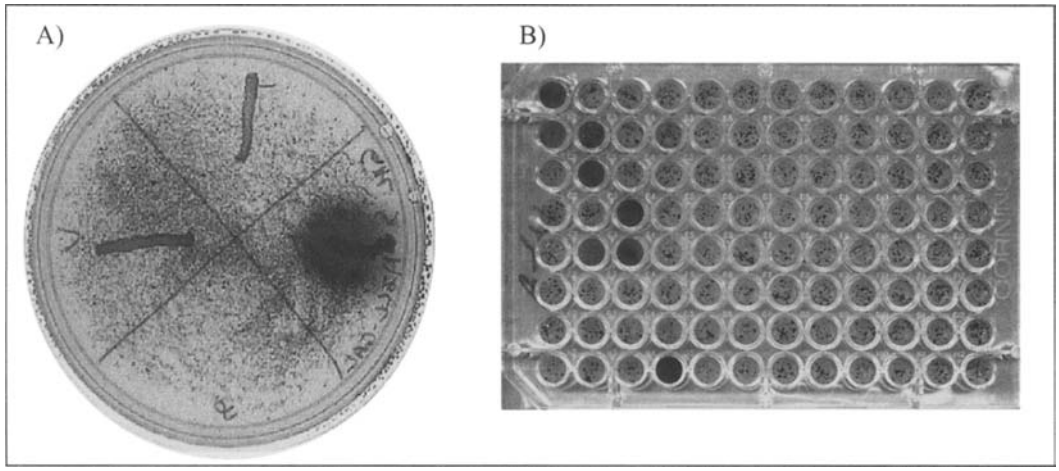


Fig. 33.23. AZCL-polysaccharide hydrolysis in (a) Petri plates and (b) microtiter plates. The dark particulates are the AZCL-β–Glucan (a) and AZCL-galactan (b). Soluble blue dye is released upon hydrolysis.

mannose from the nonreducing end of the mannose chain.[310–312] Debranching of galactomannan is primarily carried out by α-D-galactosidase (EC 3.1.2.22).[313,314] Tenkanen and co-workers have also reported an acetyl glucomannan esterase active on the acetyl side chains in glucomannan.[238,315,316] There are little other data on specific debranching enzymes involved in degradation of glucomannan. Assays for mannan hydrolysis have been carried out using extracted polysaccharides as substrates, colorimetric analogues, and dyed polysaccharides. Dyed polysaccharides have been utilized to determine activities of cultures on various polysaccharides, both as activity screens and as quantitative measures. These substrates include both soluble and insoluble forms (dependent mainly on the properties of the native polysaccharides) and include azurine (azo-), Remazol Brilliant Blue (RBB), and Ostazin Brilliant Red (OBR), among others.[317–320] For azo-, RBB-, and OBR-linked substrates, clearing zones on petri plate or acrylamide gel agar overlays indicate active colonies or protein bands.[321–325] The crosslinked version (AZCL-polysaccharides, Megazyme, Inc. Bray, Ireland) has also been used to screen for activity of various glycosyl hydrolases.[326–328] In the case of AZCL substrates, the result of activity is a blue halo surrounding active colonies or dye release into microtiter plate wells. The authors have used this technique extensively to screen both environmental samples and recombinant libraries for glycosyl hydrolase activities (Fig. 33.23).

PECTINASES

In addition to cellulose and the hemicelluloses, pectins are a third class of polysaccharides found in the cell wall matrix of plant cells. Further information and good structural diagrams can be found in the recent review by Ridley et al.[329] Found mainly between adjacent cell walls in the middle lamellae, pectins fall into three classes differentiated by their backbone structure and branching patterns.[330,331] Homogalacturonan (xylogalacturonan) is comprised of α-(1 $\rightarrow$ 4)-linked galacturonic acid chains containing xylose side chains and makes up the smooth region of pectin.[332] Homogalacturonan is methylated through ester-linkages to the galacturonic acid residues. Once in place, pectin methyl esterases (EC 3.1.1.11) remove these side chains and allow formation of the gel matrix.[333] The rhamnogalacturonans make up the "hairy" region of pectin. In rhamnogalacturonan I, (RG I) the backbone chain is comprised of the disaccharide ($\rightarrow$4)-α-D-galacturonic acid-α-(1 $\rightarrow$2)-α-L-rhamnopyranose-(1 $\rightarrow$). The rhamnose is typically substituted at the C-4 position with a

branched chain of sugars made up of either galactose or arabinose or a combination of both and other sugars. The arabinose residues can be derivatized with ferulic acid. The galacturonic acid residues in the backbone are usually *O*-2 or *O*-3 acetylated and *O*-6 methylated. The structure and substitution patterns of RG I vary widely across plant species. Where the majority of the side chain is comprised of arabinose, the side chains are referred to as arabinans. These arabinans are predominantly α-(1 $\rightarrow$ 5) linked arabinofuranosyl residues substituted at either or both the *O*-2 and *O*-3 positions.[331] Side chains comprised of galactose residues are referred to as galactans. When these galactans are further substituted with arabinan chains, they are refered to as arabinogalactans.[334] In contrast to RG I, the structure of Rhamnogalacturonan II (RG II) is highly conserved across the plant kingdom.[335] It is comprised of 28 glycosyl residues, of which seven are found in the galacturonic acid backbone. The backbone is specifically branched at four points (designated A–D chains) with some unusual sugars such as 2-*O*-methly-L-fucose and 3-deoxy-D-manno-2-octulosonic acid, aceric acid, and apiose.[335] The structures of each branch are known, although the exact point of attachment of branch D is still unclear.

As is apparent from the complex structure, there is a diverse enzyme suite required to hydrolyze pectin. As in hemicellulase systems, there are depolymerizing and debranching enzymes, mainly esterases, that act synergistically.[320,336] The depolymerizing enzymes include both glycosyl hydrolases, which cleave glycosidic bonds by an acid-base catalysis mechanism, and polysaccharide lyases, which hydrolyze the glycosidic bond through a β-elimination mechanism, resulting in a double bond between the C-4 and C-5 of the new nonreducing end.[320] Recent reviews by Kashyap et al. and by Kennedy and Methacanon outline the pectinase enzymes in detail.[143,337] Assay techniques involve the usual assortment of reducing sugar production, viscosity reduction, HPLC analysis, and dye release.[320,336,338–343] Ruthenium red staining in plates and zymograms have also been used.[336] Because of its solubility, pectin incorporated into plates can be detected by precipitation with hexadecyl-trimethyl-ammonium bromide, resulting in clear halos of hydrolysis around active colonies.[344] As with other polysaccharide degradation studies, structural determination of products has been carried out by nuclear magnetic resonance spectroscopy.[338]

CHEMICAL CATALYSIS FOR BIOMASS DECONSTRUCTION

INTRODUCTION

Deconstruction is a somewhat misleading term, in that it suggests processes exhibiting low selectivity or harsh conditions. To the contrary, *selective* biomass deconstruction can be a powerful tool for use within the biorefinery, as it provides both monomeric and polymeric materials for eventual conversion into final refinery outputs. Biomass deconstruction is a separation process highly analogous to petrochemical refining. In any process designed to convert a source of raw materials into products, the raw material almost always requires some form of preliminary upgrading in order to separate a complex mix of materials into individual streams of initial building blocks. The petrochemical industry "deconstructs" crude oil, using distillation to make mixed hydrocarbon streams for various fuel uses, or cracking (primarily using chemical catalysis) to prepare the industry's primary building blocks of ethylene, propylene, BTX, butenes, and methane for chemical uses.[345]

The biorefinery is no different. Renewable raw materials are complex mixtures of different biopolymers, and chemical deconstruction processes (as differentiated from biochemical processes described earlier in this chapter) may be used to separate these biopolymers from one another. However, deconstruction processes optimized for the biorefinery offer more flexibility than those used in the petrochemical industry. The petrochemical industry tends to focus on refining processes that give low molecular weight (very roughly <mw 300) materials. Biorefining, depending on how it is carried out, can give either high-molecular-weight

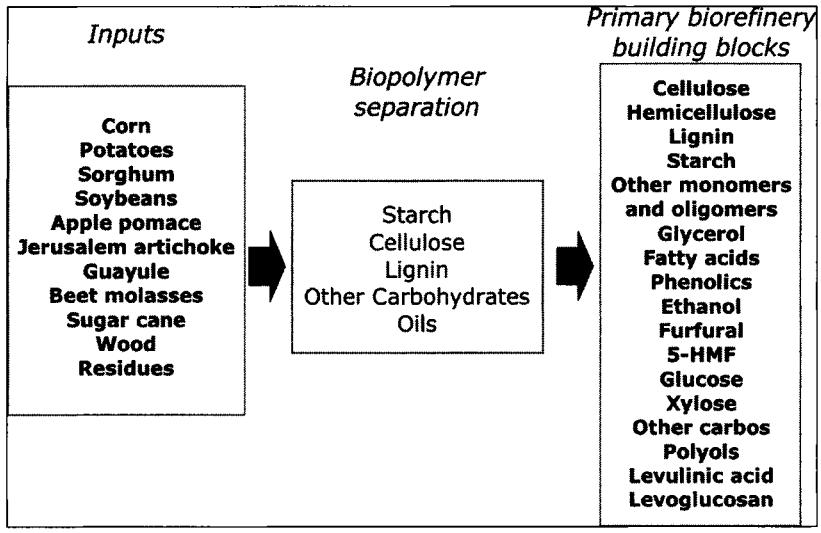


Fig. 33.24. Biomass deconstruction is a Key step in its conversion to chemical products and fuels.

biopolymers or low-molecular-weight chemicals and intermediates, both suitable for use by the chemical industry (Fig. 33.24).

The wide range of different biomass materials can be somewhat daunting. However, biomass deconstruction processes greatly simplify this initial slate of raw material sources. Biomass provides three primary feedstocks: sugars, in the form of starch, cellulose, and hemicellulose; aromatics, in the form of lignin; and hydrocarbons, in the form of plant triglycerides. Further deconstruction of these polymers and oligomers leads to monomeric materials including individual sugars and related polyols such as glycerol from triglycerides, low molecular weight aromatics from lignin, and fatty acid hydrocarbons from triglycerides.[346,347] Under proper conditions, deconstruction of the polymeric materials can also lead to structural modification of the monomeric units, leading to materials such as furfural, levulinic acid, and levoglucosan.[348]

Chemical catalysis can play a key role in each of these deconstruction processes, promoting conversions of raw materials into individual polymers, and individual polymers into low-molecular-weight compounds. This section reviews examples of each type of process, showing how catalysis leads to core building blocks for the biorefinery, analogous to the initial building blocks manufactured by the petrochemical industry. Although highly important to overall biorefinery operation, further catalytic transformations of this initial set of building blocks to chemical products is not included in this discussion.

The selective deconstruction of biomass for biorefinery use is of particular importance when viewed in the context of biomass availability. Biomass is exceedingly abundant. A vast amount of renewable carbon is produced in the biosphere, as much as 170×10^9 metric tons is fixed annually. This amount could supply almost all domestic organic chemical needs, currently about 7 to 8 percent of our total nonrenewable energy and materials consumption.[349–352] When measured in energy terms, the amount of carbon synthesized is nearly ten times the world consumption.[353] Cellulose, the most abundant organic chemical on earth, has an annual production of about 90×10^9 metric tons.[354–356] Lignin production by the pulp and paper industry is $30–50 \times 10^6$ metric tons/year.[357] The yearly availability of renewables is most important, inasmuch as—in contrast to petrochemicals—it makes this resource almost unlimited, if used in a sustainable manner. Some projections have world oil production peaking in the next 5 to 10 years.[358,359] It is unlikely that demand will decrease in line with production. In the United States, energy consumption has

increased by more than 28 percent—about 21 ekajoules (EJ)—during the last 25 years, with more than half of this energy growth—about 11 EJ—occurring during the last 6 years, indicating that other feedstock sources will be needed.[360] Chemical catalysis has been a key contributor to the success of the petrochemical industry, with more than 90 percent of all processes using some form of catalysis.[361] It is reasonable to assume that catalysis tailored for the specific features of biomass raw materials will play a similar role within the biorefinery, both to effectively and sustainably use this resource and to help address the growing needs of the world.

SCOPE OF THE REVIEW

Chemical catalysis for processes leading to cellulose, hemicellulose, lignin, and their derivatives is an extremely broad topic. It is also somewhat hard to define for biomass. A number of different processes exist for separation of biomass into its constituent biopolymers. The corn wet mill produces carbohydrates as starch. The kraft process for pulping of wood produces carbohydrates as cellulose, and under certain conditions, isolable lignin. The kraft process itself is stoichiometric, but extensive research has been done on the use of catalytic amounts of additives to promote this and related separations.[362] Many other separation processes have been categorized under the term "pretreatment"[363–366] and are linked closely to the generation of fermentable sugars for fuel ethanol production, but can also provide polymeric materials for chemical use. Some pretreatments are solvolyses, which are technically noncatalytic. Yet the active component of the solvent (e.g., a proton) is not consumed within the molecular level mechanism, and serves to promote the reaction in a catalytic sense. Other systems are autocatalyzed through generation of a catalytically active species over the course of the separation.

The subsequent deconstruction of biopolymers into single, low-molecular-weight chemicals, allows the identification and definition of catalyst to become clearer. Chemical catalysis has been used to improve many of these processes, and several examples of these conversions are described below.

The options for practical operation of a biorefinery are also complex. Very broadly, the biorefinery will have the capability of deconstructing biomass into several categories of outputs including:

- Constituent polymers of biomass
- Oligomeric fractions of these polymers
- Monomers comprising these polymers or oligomers
- Individual chemicals from structural reorganization of other fractions.

Ideally, biorefinery operation will be selective and flexible enough to switch among these categories depending on the needs of the market. Moreover, these categories could, in principle, be combined to provide diverse product profiles.[367,368] In one instance, the biorefinery would provide cellulose, hemicellulose, and lignin. However, modification of conditions could further transform the initially produced carbohydrate polymers in situ, for example, hydrolyzing the hemicellulose to xylose, and then converting it to furfural. The Acetosolv process for the separation of wood is operated in this manner.[369] Pulping of wood with the Formacell process (pulping with HOAc/HCOOH mixtures) can produce cellulose, lignin, furfural, and 5-hydroxymethylfurfural as primary products.[370] Thus the exact choice of operating mode and resulting product profile can be strictly dependent on the needs of the biorefinery operators.

Even with this potential variability in operation types, it is anticipated that the biorefinery industry will migrate toward a small group of easily produced primary building blocks from which a much larger group of final products will be derived. This approach would directly parallel the petrochemical industry, which uses seven primary building blocks for the eventual production of tens of thousands of marketplace products. The analogous group of primary building blocks for the biorefinery has not yet been defined, making the potential matrix of deconstruction processes and initial outputs complicated. Using carbohydrate polymers

again as an example, hemicellulose, xylose, or furfural could each be a biorefinery primary building block as they are all linked by chemically catalyzed deconstruction processes.

In this section, we seek to identify materials that are the reasonable first structures to arise from biomass deconstruction, and to describe how chemically catalyzed processes are being developed for their production. For that reason, commercially practiced processes that use catalysis, such as the reduction of glucose to sorbitol, are mentioned only briefly or not at all. Chemical catalysis will certainly play an additional role in the further conversion of these initial building blocks into secondary intermediates or final marketplace products (e.g., oxidative conversion of levulinic acid into succinic acid), but such multistep possibilities are outside the scope of this discussion.

SEPARATION OF BIOPOLYMERS FROM BIOMASS RAW MATERIALS

Biomass raw materials are made up primarily of the three biopolymers, cellulose, hemicellulose and lignin, and each of these materials finds use as a chemical feedstock. A wide variety of methods has been described for their separation and isolation. The paper industry describes these processes as pulping, whereas the biorefinery industry more normally refers to them as pretreatments. The goal in each case is the same: production of separate biopolymer streams for conversion to final product outputs. The uses of chemical grade cellulose are well known in industry and the literature and include cellophane and various cellulose esters and ethers.[215] Certain forms of lignin are also items of commerce.[371] Chemical catalysis of the following pretreatment/pulping processes has been examined to improve the initial separation of lignocellulosics into their constituent polymers.

Dilute Acid Pretreatments

Within the context of the biorefinery, the use of dilute acid is probably the most widely studied technology for the separation of the starting biomass raw material into its individual components. This process has been and continues to be extensively investigated, and the reader is referred to the many reviews available in the literature.[88,363–366,372–378]

Solvent Separation Processes

Biomass separation based on the use of organic solvents ("organosolv" processes) offers more selective access to the biopolymeric constituents of renewable raw materials, as such operations normally give separate and high-quality process streams of cellulose, hemicellulose, and lignin. Several reviews on organosolv technology and the mechanism of separation are available.[379–385] Most examples of organosolv processes use volatile alcohols, such as methanol and ethanol, however, a huge variety of additional solvents has been employed, including 1,3-BDO, 1,4-BDO,[386] ethylene glycol,[387] glycerol,[388] phenol,[389] and organic acids under the names of Formacell, Milox, and Acetocell.[390–395] Attempts to commercialize organosolv technologies have also appeared as the Alcell, Organocell, or ASAM processes.[385,396–402] Because these processes were primarily focused on new methods of producing cellulose pulp for the paper industry, their expense precluded commercialization. However, the selectivity of organosolv technology makes it well suited for the production of chemical building blocks from renewable raw materials.

In almost all organosolv processes, chemical catalysis plays a necessary role, as solvents alone do not function effectively for the separation of biomass.[403] The most commonly employed processes, based on the treatment of biomass with aqueous alcohols at elevated temperatures, are autocatalyzed. Acetic acid is generated during the separation process through hydrolysis of acetate groups present on the hemicellulose polymer.[397,404–406] Alternatively, acid can be added to the separation medium prior to the process. Adding acid catalyst normally allows lower separation temperatures and milder conditions. Chemical catalysis has proven to be of particular importance for the

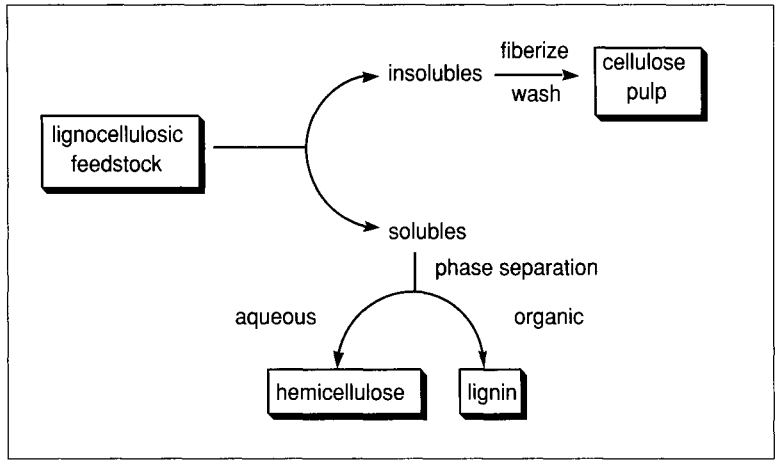


Fig. 33.25. The NREL clean fractionation process.

organosolv separation of softwoods. Normally, softwoods are poor substrates in solvent-based processes. However, the addition of alkaline earth metals to alcohol separation processes has resulted in effective softwood separation.[403,407–409]

An example of an acid-catalyzed organosolv biomass separation is the clean fractionation (CF) process, developed at the National Renewable Energy Laboratory (Fig. 33.25).[410] The process treats the biomass with a ternary mixture of methyl isobutyl ketone (MIBK), ethanol (EtOH), and water (H_2O) in the presence of sulfuric acid. The separation is carried out using a 16/34/50 ratio of MIBK/EtOH/H_2O for 56 minutes at 140°C in the presence of 0.025 M–0.2 M H_2SO_4 as standard conditions. The solvent mixture selectively dissolves the lignin and hemicellulose components, leaving the cellulose as an undissolved material that can be washed, fiberized, and further purified. The soluble fraction containing the lignin and hemicellulose is treated with water, causing a phase separation yielding an organic phase containing the lignin and an aqueous phase containing the hemicellulose. Solvent removal gives CF lignin in high yield as a shiny, brown, free-flowing powder exhibiting properties consistent with other organosolv lignins, and a generally lower-molecular-weight profile and greater solubility in polar organic solvents than other lignins. CF appears to be a very general process, and can be used for fractionation of a wide range of biomass feedstocks including corn stover, newsprint, bagasse, corn fiber, and rice straw.

Steam Explosion and Related Processes

The steam explosion process is a rapid and decades-old treatment process for lignocellulose that releases individual biomass components through steam impregnation under pressure, followed by a rapid pressure release. Steam explosion is carried out using high-pressure steam and short contact times.[411–414] This process has generally been used as a method for preparing cellulose pulp but can also serve as source of lignin. Alkaline extraction after explosion recovers more than 90 percent of the lignin when wood is used as the feedstock.[415–419]

Catalysis of steam explosion processes has been carried out, primarily through the addition of acid[376,378,420,421] or SO_2 to the separation. Added SO_2 has been shown to exhibit benefits in conversion yields and separation conditions.[422,423] The characteristics of the oligosaccharides and lignin from these processes have been examined.[415–419,424]

The Kraft Process

Kraft pulping is the most widely used process in the world for separating cellulose from wood, but is not normally considered as a potential operating unit for biorefineries. Certainly for

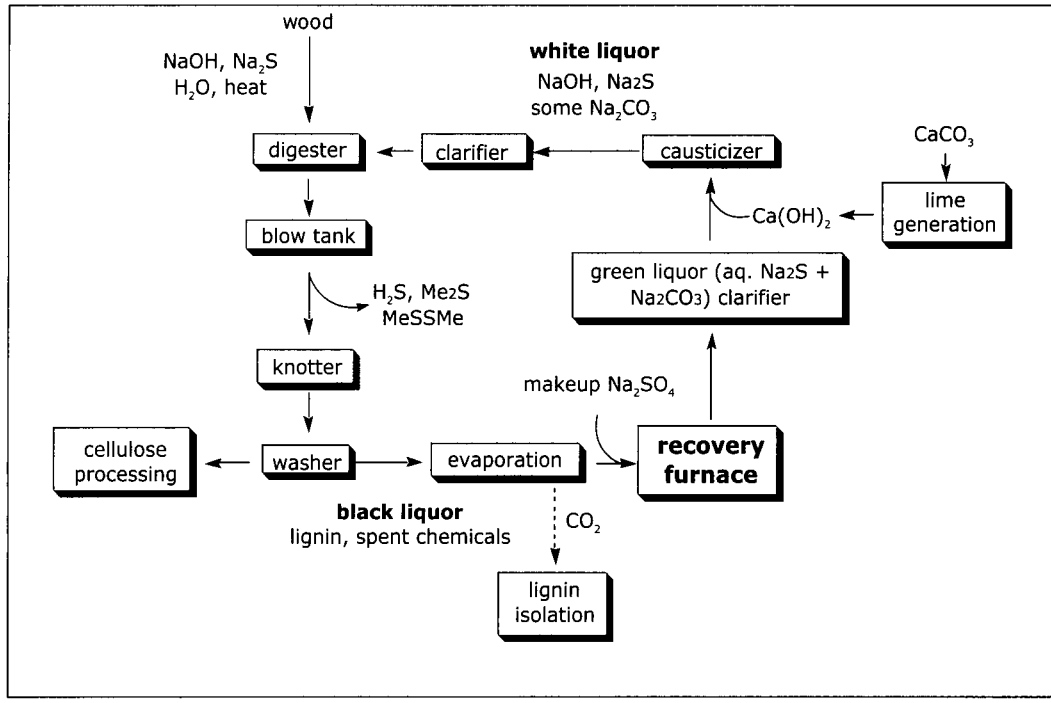
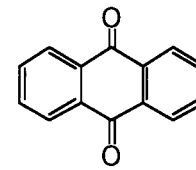


Fig. 33.26. Overview of the kraft process.

new construction, use of the kraft process would face considerable challenges, including the enormous capital cost of a new plant. Nonetheless, the existing infrastructure of the pulp and paper industry consumes more than 100×10^6 metric tons/yr of wood, the majority of this with kraft technology, offering a possible opportunity for any underused capacity.[103]

The process is conducted at the high pH in the presence of sodium sulfide, sodium hydrosulfide, and sodium hydroxide at 150–180°C for about 2 hours (Fig. 33.26). Along with the primary cellulose product, the process generates a solution containing dissolved lignin, most of the wood's hemicelluloses, and spent pulping chemicals ("black liquor"). This solution is used in the kraft recovery boiler as a source of power for the mill and fuel for chemical recovery operations crucial to economic operation. The chemistry of pulping reactions has been the subject of much study, and summaries are available the literature.[425–427]

The kraft process itself is stoichiometric, however, the process can be improved through the addition of catalytic amounts of anthraquinone (AQ, **1**). The use of AQ in alkaline pulping processes has been widely examined by the industry.[362,428,429]



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As a catalyst, AQ offers a number of benefits, including an increase in pulping rate, a decrease in the amount of pulping chemicals used, a decrease in degradation of the cellulose during pulping, and improvement of cellulose bleaching processes. In the context of the biorefinery, these improvements lead to faster production and higher yield of a more easily purified cellulose biopolymer. AQ is typically added at levels of 0.05 to 0.1 percent based on the weight of raw wood feedstock used. Considerable mechanistic study has been performed to determine how AQ exerts these effects.[430,431]

DECONSTRUCTION OF BIOREFINERY RAW MATERIALS AND BIOPOLYMERS TO MONOMERIC PRODUCTS

Initial separation of biomass raw materials can yield separate streams of biopolymers, each of which has potential utility as a product within the biorefinery. Further selective deconstruction processes can convert these biopolymers into their individual monomeric units, or to structurally rearranged materials. The following are examples of processes that have been improved through the use of chemical catalysis.

Polymeric Carbohydrate Hydrolysis Processes

The hydrolysis of polysaccharides into monomeric sugars is a well-studied process; its mechanism is still believed to be accurately described by the work of Saeman in 1945.[432] Its significance is linked to the production of fermentable sugars for ethanol manufacture. Many of these processes are based on acid catalysis and overlap closely the pretreatment methods described earlier.[363,364,373] Treatments with both concentrated and dilute acids are well known as methods for the hydrolysis of cellulose and hemicellulose, as is the use of organic dicarboxylic acids as alternative catalysts to mineral acids.[433]

However, further cellulose deconstruction is considerably more difficult after acid hydrolysis. Commercial processes for acid hydrolysis of cellulose were known in the early part of the twentieth century.[434] The use of either low- or high-acid concentrations has disadvantages. At high concentrations, corrosion-resistant reactors and an effective acid recovery process are needed, raising the cost of the intermediate glucose. Dilute acid treatments minimize these problems, but a number of kinetic models indicate that the maximum conversion of cellulose to glucose under these conditions is 65 to 70 percent because subsequent degradation reactions of the glucose to HMF and levulinic acid take place. The modern biorefinery is learning to exploit this reaction manifold, because these "decomposition" products can be manufactured as the primary product of polysaccharide hydrolysis (see below).

Carbohydrate Pyrolysis

Pyrolysis of C6 mono- and polysaccharides deconstructs the polymer chain forming either levoglucosan or levoglucosenone. In the presence of an acid catalyst (normally H_3PO_4), levoglucosenone is also formed in low yields (Fig. 33.27).[435–440] The transformations are straightforward, but separation of these potential biorefinery building blocks is complicated by the formation of considerable amounts of tar. Nonetheless, attempts have been made to develop levoglucosan as a commercial product.[348]

The formation of levoglucosan and levoglucosenone is found to be strongly dependent on the presence of catalytic amounts of various cations during pyrolysis. However, the literature is inconsistent regarding the effect of these cations. By varying the amount of acid catalyst used, Faix et al. were able to control the relative amount of the two products.[441,442]

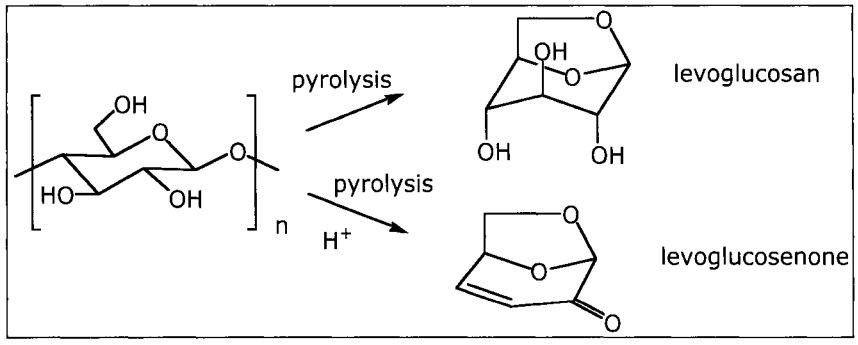
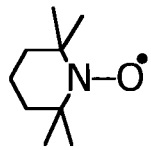


Fig. 33.27. Production of levoglucosan and levoglucosenone.

Transition metal catalysts were also found to influence the course of the pyrolysis. Addition of Fe or Cu increased the yield of levoglucosan formation from wood or newsprint.[443] The presence of Ca ions also increased the yield of these products, whereas catalytic amounts of Na ions were found to decrease the pyrolysis yields.[444] In contrast, earlier work indicated that Li, K, and Ca ions all had a negative effect on the pyrolysis process.[445]

Carbohydrate Oxidation

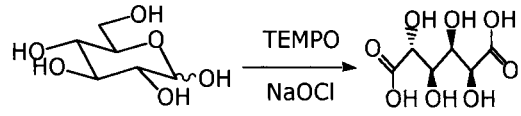
A considerable amount of recent work has focused on the oxidation of polymeric and monomeric carbohydrates in aqueous media. In the context of the biorefinery, these processes could be used for the preparation of oxidized carbohydrates as primary outputs of biomass deconstruction. Of particular interest are processes catalyzed with stable oxygen-centered radicals such as the nitroxyl radical TEMPO (2) (2,2,6,6-tetramethylpiperidinoxyl) and using bleach as the stoichiometric oxidant.



TEMPO, **2**

The reaction appears to be well suited for selective conversion of biomass carbohydrates into their corresponding oxidized derivatives. This system serves to oxidize several polymeric carbohydrates including starches and pullulan.[446–449] More recent work has described the TEMPO-catalyzed introduction of carboxyl groups in native cellulose and its different morphological forms,[450–452] cellulose derivatives,[453] and the surface of cellulose nanocrystals.[454] The related biopolymer, chitin, also is oxidized under these conditions.[455]

Under proper conditions, biomass deconstruction will generate streams of monomeric or oligomeric carbohydrates, with glucose and xylose (from cellulose/starch or hemicellulose, respectively) being the most likely initial feedstocks. Glucose oxidation in the presence of TEMPO and bleach has been used for the production of glucaric acids (see below) by selective, high-yield oxidation of the initial and terminal carbons of the starting sugar.[456–459]



D-Glucose Glucaric acid

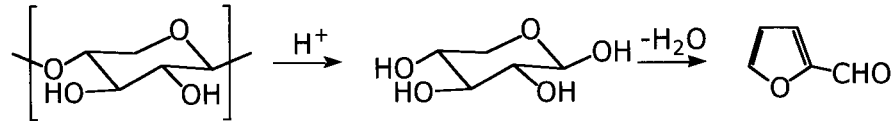
These materials can serve as novel polymer components in the production of new polyhydroxylated polyamides[460] and as starting points for the production of other biorefinery intermediates. Conventional processes for the conversion of sugars to aldaric acids use HNO_3 as the oxidizing agent. However, yields of aldaric acids using these processes are only fair, and the use of HNO_3 as the oxidant is potentially hazardous and environmentally unfriendly (NO_x emissions). Development of single-step biorefinery processes could make aldaric acids a useful primary building block of the biorefinery.

Other carbohydrates can also undergo selective, high-yield oxidation. For example, sucrose (the primary product of the sugar industry) converts into the corresponding tricarboxylic acid.[461]

Carbohydrate Dehydration

The removal of water from initially formed biomass sugars is an important process for the production of primary biorefinery building blocks. Of particular interest are 5-hydroxymethylfurfural and levulinic acid (from the dehydration of glucose or other C6 sugars) and furfural (from xylose dehydration). Recent research has led to new catalytic processes for the production of each of these materials.

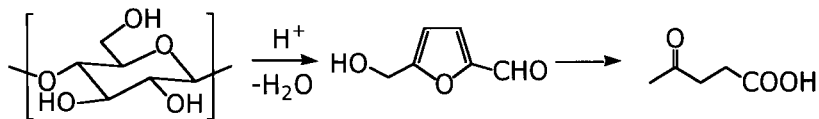
Furfural. Dehydration of xylose, available from biorefinery hemicellulose, leads to the production of furfural.[462–464]



Although the process for furfural production has been known for decades and is applicable to most any C5-sugar-containing feedstock,[465–468] it is not highly efficient, with commercial yields generally around 50 percent.[469] Current research is directed at improving the production of furfural from xylose or hemicellulose with new catalytic transformations. Improvement of process conditions for the standard H_2SO_4-catalyzed furfural production from the xylose in corn cobs was realized by use of HCl, leading to an 85 percent furfural yield.[470] A related process charges dry feedstock with catalytic gaseous HCl, minimizing decomposition reactions through a more rapid removal of furfural from the reactor.[471]

conversions.[475] Faujasite and mordenite catalysts have been examined as acid catalysts.[476] The presence of metal oxides has also been reported to improve furfural yield.[466]

Levulinic Acid. Dehydration of glucose or other monomeric and polymeric C6 sugars leads to the direct formation of levulinic acid (LA) as a potential primary building block for the biorefinery, and several reviews have described its potential commercial utility.[477,478] The preparation of levulinic acid is not difficult, although the mechanism of its formation from carbohydrates is complex, and offers several alternative decomposition pathways (equation 3).[479]



The need to recover homogeneous acid catalysts in the production of furfural has spurred the investigation of acidic heterogeneous catalysts to promote the dehydration of C5 sugars. A relatively high conversion of xylose to furfural was reported using heterogeneous sulfonated mesoporous silicas.[472] Rapid removal of furfural from the reaction mixture using supercritical CO_2 has been examined as a method to improve furfural yields.[473,474] Combining supercritical CO_2 extraction with xylose dehydration in the presence of sulfated titania and zirconia catalysts, led to high furfural yields at high xylose

Dehydration of glucose in the presence of microporous pillared clays has also been explored but, the selectivity to levulinic acid was low.[480]

The most widely used approach for producing levulinic acid is the direct hydrolysis of biomass or carbohydrates, catalyzed by aqueous acid.[478,480–483] However, this forms large amounts of side products and intractable materials, requiring an expensive separation that drives up its cost. Several years ago, a new process developed by the Biofine Corporation eliminated many of the existing production problems. The Biofine process

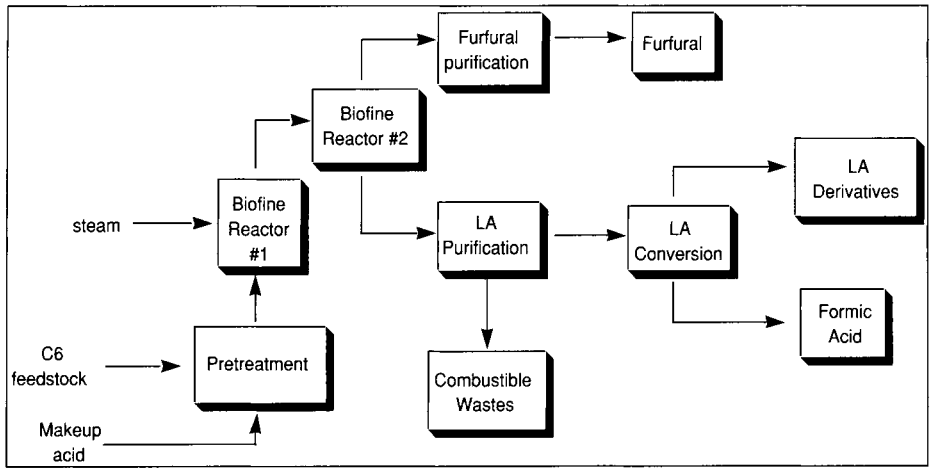


Fig. 33.28. The Biofine process.

also uses acid hydrolysis of carbohydrate sources (cellulose, starch, paper mill sludge, urban waste paper, agricultural residues, or paper fines) to produce LA, but in a novel, two-reactor system that minimizes sideproduct formation and associated separation problems (Fig. 33.28).[484,485]

The process supplies carbohydrate-containing materials to a first reactor where they are briefly hydrolyzed at 210–230°C in the presence of mineral acid. This initial hydrolysis produces hydroxymethylfurfural, which is removed continuously and supplied to a second reactor. In the second reactor, the hydroxymethylfurfural is hydrolyzed further at 195–215°C for 15–30 minutes to produce levulinic acid, which is again continuously removed. The yield of LA is 75 percent or greater, based on the C6 content of the carbohydrate containing starting material, one of the highest reported. Two real strengths of this process are its relative insensitivity to the type of feedstock employed and the wide availability of feedstock sources. Any material containing C6 sugars will serve as a good starting material. The result is a cost-effective process, making it suitable as a starting material for a wide variety of products. Economic projections indicate that the LA production cost using this process could be as low as \$0.08–\$0.12/lb depending on the scale of the operation. This cost is consistent with the use of levulinic acid as a primary biorefinery building block.

5-hydroxymethylfurfural (5-HMF). Dehydration of C6 sugars also produces 5-HMF (shown above), which is an intermediate within the dehydration mechanism leading to levulinic acid. 5-HMF has been suggested as a potential primary building block for the biorefinery,[486–488] but its relatively high instability and associated difficulties in its isolation have hindered its development.

Polymeric carbohydrates available from the biorefinery can serve as starting materials for 5-HMF. Recently, $LaCl_3$ has been used to catalyze the conversion of cellulose to 5-HMF (along with glucose, levulinic acid, and cellobiose) at elevated temperatures in water.[489]

A primary reason for low yields of 5-HMF is its rapid conversion to levulinic acid in aqueous media. However, catalysis of the transformation with lanthanides has led to dramatic increases in the yield of 5-HMF. The effectiveness of different lanthanide cations has been surveyed.[490,491] In nonaqueous solvents, such as DMSO, almost quantitative yields of 5-HMF have been reported.[492] Performing the dehydration in the presence of activated carbon (to adsorb the generated 5-HMF) has also been reported as effective.[493]

Recent work has investigated the selective catalytic dehydration of fructose (available

from corn starch) and related sugars such as glucose, sucrose, and inulin as a source of 5-HMF. Standard preparation of 5-HMF is carried out using aqueous acid catalysts.[494–496] The process can be improved by dehydrating the fructose with an Amberlyst catalyst in an ionic liquid as the reaction medium. Other solid acid catalysts have been reported.[497–499] Sub- and supercritical acetone has also been used as an alternative medium to improve 5-HMF production.[500] 5-HMF yields of 80 percent were reported under these conditions in comparison to control reaction yields of only 50 percent.[501] A number of heterogeneous catalysts have been used to promote this transformation.[483] The use of vanadyl phosphate doped with trivalent metal cations has been reported as effective for the production of 5-HMF in high yield without the formation of polymeric byproducts.[502] Niobium phosphate catalysts have also been investigated,[503,504] as well as acidic mordenites.[505] A mechanism for the catalytic reaction over zeolites has also been suggested.[506]

Lignin Deconstruction

Deconstruction of lignin has been carried out for over a century, but in a largely nonselective manner. There is no doubt that lignin is widely available. It contributes as much as 30 percent to the weight of lignocellulosic biomass, making it second only to cellulose as a source of renewable carbon. Because lignocellulosic will be a major raw material source available to the biorefinery, isolation of sugars for use in one operating unit of the biorefinery guarantees a parallel lignin process stream. Moreover, the separation processes described earlier will provide separate carbohydrate and lignin components. Lignin's native structure (a complex network of oxygenated aromatic rings) certainly suggests that it could play a central role as a new chemical feedstock. But today's reality is that lignin is routinely relegated to very low value uses when considered as a biorefinery process stream. In the pulp and paper industry, the bulk of the removed lignin is used as a boiler fuel for chemical recovery. In other parts of the process, elegant bleaching sequences have been developed for the sole purpose of nonselectively converting residual lignin remaining with the cellulose into small, low-molecular-weight materials that are easily washed out.[507] Catalysis is playing a role in these processes.[508–518] Nonetheless, more selective catalytic lignin conversion processes continue to be examined.[519]

General Lignin Oxidation Processes. A vast amount of work has been done on lignin oxidation, primarily to understand and develop new pulp bleaching processes. More recently, the use of catalytic processes has been examined. If made selective, these types of processes could yield a broader family of lignin-derived primary building blocks. Because of the complexity of lignin's structure, much of the actual research has been carried out using lignin models. The oxidation of lignin models with HOOH in the presence of Co catalysts has been studied.[520,521]

Several more selective oxidation processes have been examined for the conversion of lignin to biorefinery building blocks. Oxidation of lignin residue from dilute acid pretreatment leads to a stream of mixed aromatic aldehydes.[522] Cu-catalyzed lignin oxidation with oxygen leads to the production of monomeric phenols.[523] Improved separation technology can allow effective use of these mixed streams within the biorefinery.[524,525] Modeling has shown lignin conversion into quinones using cobalt and heteropolyacid catalysts.[526–528] and aromatic aldehydes with catalytic NO_2 in the presence of oxygen.[529]

Phenol Hydrogenation. In principle, appropriate lignin deconstruction processes will provide a stream of mixed phenols. Reduction of these phenols will lead to a new source of cyclic aliphatic alcohols of potential use in the manufacture of adipic acid derivatives. Several catalytic processes for these types of reductions have appeared for phenol and should be applicable to lignin-derived mixed phenols. Phenol itself is reduced to cyclohexanol in the presence of various heterogeneous catalysts based on Pd.[530–535]

Vanillin. Catalytic oxidation of lignin has long been used as a source of vanillin. For the

most part, yields in these processes tend to be very low, generally less than 10 percent. However, vanillin production can be used to provide a low-volume, high-value revenue stream within certain lignin-generating operations.[536,537] The mechanism of the transformation has been reported.[538–542]

CONCLUSIONS

Deconstruction of biomass raw materials offers the biorefinery access to a large number of both polymeric and monomeric primary building blocks. Biomass offers significant flexibility as to the product slate that various separation processes may realize. These materials, which are the biorefinery analogues to ethylene, propylene, BTX, and other building block chemicals of the petrochemical refinery, will serve as the platforms from which much larger families of products can be produced. Importantly, these processes also offer access to a wide range of new structures that can complement those currently available from the petrochemical industry. The challenge in using these materials will be development of appropriate technology tailored to accommodate these structures and facilitate their conversion into biorefinery intermediates and final products.

THERMOCHEMICAL BIOMASS CONVERSION

INTRODUCTION

Biomass combustion has been a source of heat and light for thousands of years and is one of the oldest examples of thermochemical biomass conversion. More recently, biomass thermochemical conversion processes and technologies are receiving renewed attention as concerns about the sustainability of energy resources increase and mounting evidence of global climate change brought about by fossil fuel consumption continues to be revealed. Advances in crop production and harvesting, collection, and use of biomass from thinning operations for improved forest health, and utilization of biomass residues can provide lower-cost biomass feedstocks. Integrating lower-cost feedstocks with advances in higher-efficiency biomass thermochemical conversion processes for the production of fuels, chemicals, and combined heat and power, provides a number of realizable social, economic, energy, and infrastructure security benefits for the future.[543–545]

As defined above, biomass materials can be quite varied with a wide range of physical, fuel, and chemical properties. This variability in the inherent properties of biomass resources determines conversion technology options that are appropriate for specific applications.

Thermochemical conversion applications are generally fuel-flexible when it comes to variations in biomass composition. The main biomass fuel properties that affect the long-term technical and economic success of a thermochemical conversion process are moisture content, fixed carbon and volatiles content, impurity (S, N, Cl) concentrations, and ash content. Collectively, these properties affect the energy density of biomass fuels. The higher heating value of biomass on a dry and ash-free basis is typically around 8000–9000 Btu/lb (18.5–20 MJ/kg). High moisture and ash contents reduce the usable energy of biomass fuels proportionally. Therefore, from an energy perspective, maximum system efficiencies are possible with dry, low-ash biomass fuels. However, drying biomass can be costly and low-ash biomass resources are generally considered premium fuels that tend to be more expensive.

The bulk density of biomass feedstocks is also quite low compared to traditional fossil fuels. Lower bulk densities and lower energy densities translate into higher costs for feedstock preparation, handling, and transportation, putting biomass conversion technologies at an economic disadvantage in current energy markets. Consequently, biomass utilization is most advantageous when the feedstock is either a high-volume waste product of an existing system or is readily available in close proximity to the conversion plant. Examples of this can be found in the pulp and paper and wood products industries. In fact, the pulp and

TABLE 33.5 Proximate and Ultimate Analyses of Selected Biomass Feedstocks

	Eucalyptus	*Poplar*	*Willow*	*Switchgrass*	*Rice Straw*	*Wheat Straw*	*Corn Stover*	*Almond Shells*	*Alfalfa Stems*	*Black Thunder Coal*	*Pittsburgh #8 Coal*
HHV, MJ/kg, dry	19.18	19.46	19.34	18.36	14.71	17.64	18.06	19.01	18.63	29.75	31.80
Proximate wt% as received											
Ash	0.48	1.16	0.85	4.22	17.30	6.55	4.75	2.80	4.78	5.08	7.90
Volatile	78.52	80.99	76.52	72.73	65.62	75.54	75.96	70.13	71.59	42.70	36.80
Fixed Carbon	11.66	13.05	12.40	14.89	14.23	16.22	13.23	19.22	14.34	30.91	1.14
Moisture	9.34	4.80	10.23	8.16	7.35	6.58	6.06	7.85	9.29	21.30	54.16
Ultimate wt% as received											
Moisture	9.34	4.80	10.23	8.16	7.35	6.58	6.06	7.85	9.29	21.30	1.14
C	44.89	47.05	44.07	43.04	35.42	41.96	43.98	46.20	42.79	55.01	78.02
H	5.21	5.71	5.29	5.37	4.82	5.09	5.39	5.48	5.44	3.97	4.87
O*	39.92	41.01	39.21	38.58	34.14	39.28	39.10	36.94	35.09	0.74	1.36
N	0.13	0.22	0.32	0.53	0.81	0.40	0.62	0.68	2.43	0.38	2.78
S	0.03	0.05	0.03	0.10	0.17	0.14	0.10	0.03	0.18	13.45	3.93
Cl**	0.05	<0.01	<0.01	0.46	0.54	0.21	0.25	0.01	0.45	0.06	0.09

paper industry is the largest generator and consumer of biomass energy in the form of process heat and electricity produced from black liquor recovery boilers and bark/hog fuel boilers. Agricultural practices for grain production also provide the potential to use localized residues such as straws and stover in biomass thermochemical processes. In the future, dedicated energy crops such as switchgrass, poplar, and willow could potentially provide a renewable sustainable source of fuel for thermochemical conversion processes.

Important biomass fuel properties for thermochemical conversion processes are reported as proximate and ultimate analyses. The proximate and ultimate analyses for selected biomass feedstocks are presented in Table 33.5. For comparison, the analyses from two selected coal samples are also presented. Biomass generally has a lower energy density than coal, oils, and natural gas; it also has higher oxygen content. The higher volatiles and oxygen content of biomass translate into a higher reactivity compared to traditional fossil fuels. In terms of thermochemical conversions, this means that less severe process conditions (lower temperature and shorter residence time) are required for biomass-fueled systems. The chemical composition of biomass ash and the concentration of S, N, and Cl in the biomass can have detrimental impacts on long-term system operability and environmental performance.

Biomass can be used in thermochemical processes to produce heat and electricity, as with other renewable energy technologies. However, biomass resources are uniquely suited for conversion into liquid transportation fuels, chemicals, and materials. Thermochemical conversion of biomass into heat and power, transportation fuels, and chemical feedstocks is achieved by using one of three processes: pyrolysis, gasification, or combustion. All of these processes effectively use thermal energy to dehydrate, devolatilize, depolymerize, and oxidize, partially or completely, lignocellulosic materials to varying degrees. These thermochemical conversion processes are arranged in Fig. 33.29 in terms of process severity that can be considered a function of temperature and residence time. Increased temperature also correlates with increasing oxidation, especially for autothermal processes. Pressure does not necessarily affect process severity but can affect the product composition of thermochemical conversion.

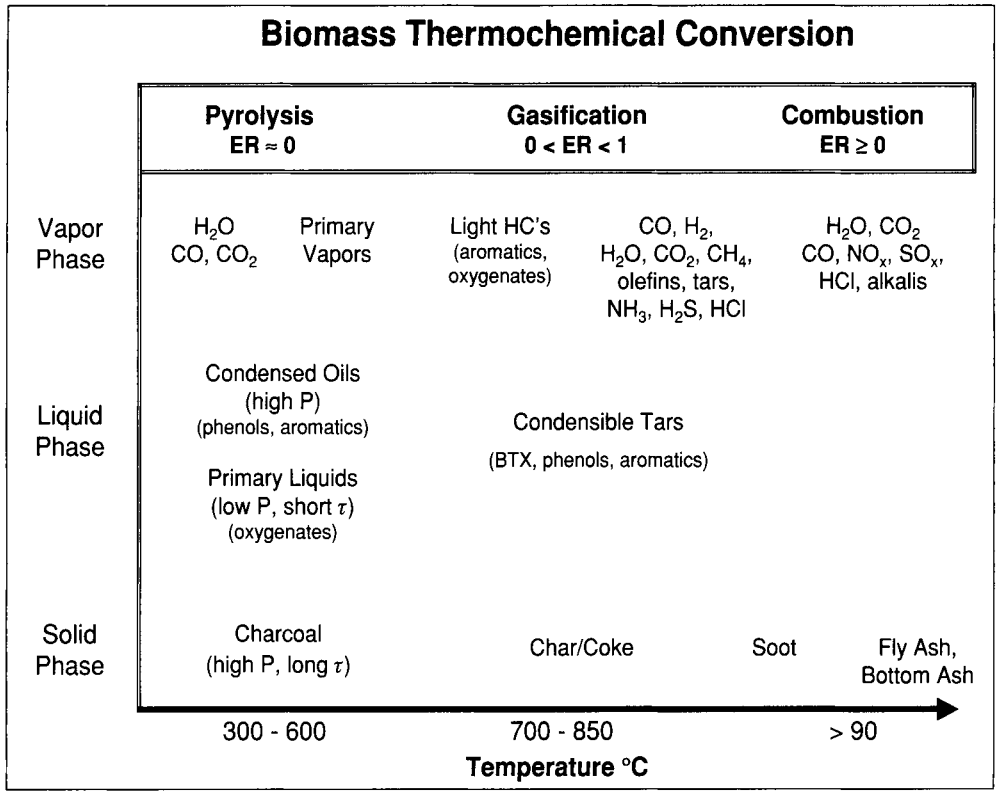


Fig. 33.29. General product composition as a function of process severity for biomass thermochemical processes.

Biomass pyrolysis is the thermal depolymerization of biomass at modest temperatures in the absence of added oxygen. The slate of products from biomass pyrolysis depends on the process temperature, pressure, and residence time of the liberated pyrolysis vapors.[546–549] Charcoal yields of up to 35 percent[550] can be achieved for slow pyrolysis at low temperature, high pressure, and long residence time. Flash pyrolysis is used to optimize the liquid products in an oil known as biocrude or bio-oil.[548] High heating rates and short residence times enable rapid biomass pyrolysis while minimizing vapor cracking to optimize liquid product yields with up to 80 percent efficiency. Hydrothermal upgrading is a related process that produces a bio-oil product that converts wet biomass at high pressures into oxygenated hydrocarbons. Biomass liquefaction converts wet biomass feedstocks into liquid hydrocarbons at low temperatures in a high-pressure hydrogen environment.

Biomass gasification consists of several elementary steps that occur during the partial oxidation of biomass by a gasifying agent, generally air, oxygen, or steam. Initial heating of the biomass leads to evaporation of water. A further increase in temperature initiates biomass pyrolysis followed by the partial oxidation of pyrolysis vapors. The char remaining after a biomass particle is devolatilized is also gasified. The biomass gasification product gas is a low- to medium-energy content gas (depending on the gasifying agent), known as synthesis gas or syngas, that consists mainly of CO, H_2, CO_2, H_2O, N_2, CH_4, and other hydrocarbons. Minor components of the product gas include tars, sulfur and nitrogen oxides, alkali metals, and particulates. These minor components of the product gas potentially threaten the successful application of downstream syngas utilization processes. Gas composition and quality are dependent on a wide range of factors including feedstock composition, type of gasification

reactor, gasification agents, stoichiometry, temperature, pressure, and the presence or lack of catalysts.

Biomass combustion systems range from very small-scale home heating applications (wood and pellet stoves) to small-scale commercial heating applications (furnaces and boilers) to modest-size biomass power plants (20–50 MW_e) to large-scale recovery boilers (400 MW_{th}) used in the pulp and paper industry. Direct biomass combustion systems can be used to generate process heat and electricity through traditional Rankine steam cycles at relatively low (17–25%) thermal conversion efficiencies. Biomass can also be cocombusted with coal to take advantage of the high efficiency of large-scale pulverized-coal-fired power plants. Biomass combustion processes are generally feasible only if feedstocks contain less than ~50 percent moisture.

Each of these thermochemical conversion processes has unique technical barriers and challenges that have been overcome with varying degrees of technical and economic success. The applications of these thermochemical conversion technologies are also in various stages of commercial application. For example, biomass combustion and biomass pyrolysis for production of a specific food additive are commercially practiced. Other technologies such as biomass gasification and use in integrated combined cycles for power production are in the demonstration stage. The technical details and challenges facing present and future application of these thermochemical biomass conversion processes are discussed in the following sections.

PYROLYSIS

Pyrolysis as defined is a process of thermal decomposition occurring in the absence of oxygen. Pyrolysis of biomass is a complicated multistage reaction for which many pathways and mechanisms have been proposed.[551–558] The best known is the model developed by Broido and Shafizadeh[559,560] for pyrolysis of cellulose that can be also applied, at least qualitatively, to whole biomass (Fig. 33.30).

As shown in this model, pyrolysis of cellulose always results in solid, liquid, and gaseous products. However, the proportions of the product yields can change depending on the process conditions. The knowledge of thermodynamics and kinetics of the reaction pathways allows us to adjust the conditions to maximize the yield of desired products. Dehydration of cellulose is exothermic, whereas depolymerization and secondary vapor cracking are endothermic and have higher activation energy than dehydration. Therefore, lower process temperature and longer vapor residence times will favor the production of charcoal. High temperature and longer residence time will increase the biomass conversion to gas, and moderate temperature and short vapor residence time, necessary to minimize secondary cracking, are optimum for producing liquids. Table 33.6 provides data on

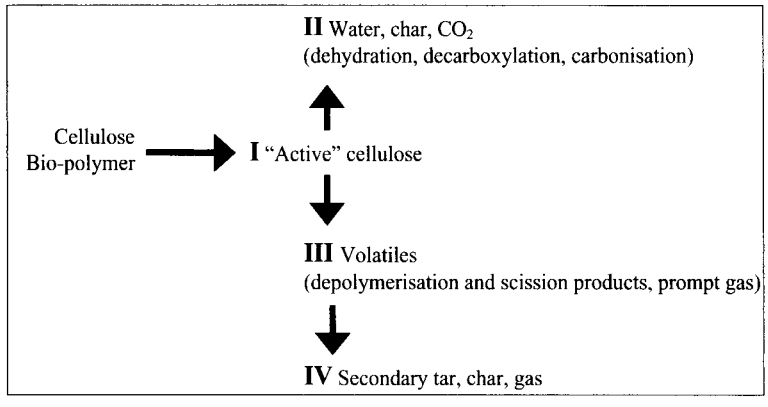


Fig. 33.30. Broido and Shafizadeh model for pyrolysis of cellulose.

TABLE 33.6 Modes of Pyrolysis and Typical Products of Wood Pyrolysis

	Liquid %	*Char* %	*Gas* %
Fast Pyrolysis Moderate temperature and short residence time, particularly vapor	75	12	13
Carbonization Low temperature and long residence time	30	35	35
Gasification High temperature and long residence times	5	10	85

the product distribution obtained by different modes of pyrolysis process.

Pyrolysis is always the first step in biomass thermochemical processes in which it is followed by total or partial oxidation of the primary products. Pyrolysis of biomass has been practiced for many thousand years to produce charcoal. In 1996, the world annual charcoal production was estimated at 100 million tons.[550,561] Charcoal is a very important fuel in developing countries and is still a highly desired reductant in the metallurgical industry because of its low sulfur and mercury content. Large amounts of charcoal are also used to produce activated carbons extensively used for cleaning water and air. Charcoal is produced by a slow pyrolysis process occurring at temperature 350–450°C with low biomass heating rates (1–10°C/min). In most processes, part of the raw material is oxidized, using a limited air access, to provide heat for the process. Although charcoal formation reactions are slightly exothermic, heat is needed for drying biomass, which always contains moisture. Existing charcoal plants range from earth mound kilns to retorts operating in batch mode (cycles from 20 days to 24 hours) to continuous shaft reactors having throughput up to 30 tons/day. Depending on the production method, the yields of charcoal can vary from 10 to 35 percent based on dry wood. In the more developed processes (Degussa, Lambiotte, Lurgi) byproducts such as acetic acid, methanol, and other chemicals are also recovered. The technologies for producing charcoal have been reviewed in many textbooks[562] and a comprehensive review of the fundamentals of slow pyrolysis processes was recently published.[550]

Fast Pyrolysis

Fast pyrolysis is one of the most recent renewable energy processes developed to maximize the production of the liquid product, bio-oil. It has achieved commercial status for production of chemicals and is very close to commercialization for the production of liquid fuels. Although related to the traditional slow pyrolysis processes for making charcoal, fast pyrolysis is carried out at a higher temperature (450–550°C) and with much higher biomass heating rates (100–1000°C/s). As a result, biomass decomposes to generate mostly vapors, gases, and aerosols, and less charcoal. After cooling and condensation of the volatiles, a dark brown liquid is formed that has a heating value about half that of conventional fuel oil.

The essential features of a fast pyrolysis process are:

- Very high heating and heat transfer rates, usually requiring a finely ground biomass feed
- Carefully controlled pyrolysis reaction temperature of around 500°C in the vapor phase, with short vapor residence times of typically less than 2 seconds
- Rapid cooling of the pyrolysis vapors to give the bio-oil product.

The main product, bio-oil, is obtained in yields of up to 75 percent wt on dry feed basis, together with byproduct char and gas, which are used within the process, so there are no waste streams. Several reviews on fast pyrolysis have been published in recent years that cover, the production, utilization, storage, and upgrading of biomass pyrolysis oils.[546,547,549,563–570]

A wide range of reactors such as bubbling and circulating fluidized beds, ablative, entrainment, rotating cone, auger, and vacuum have been operated. Fluidized beds were the most often used because of their ease of

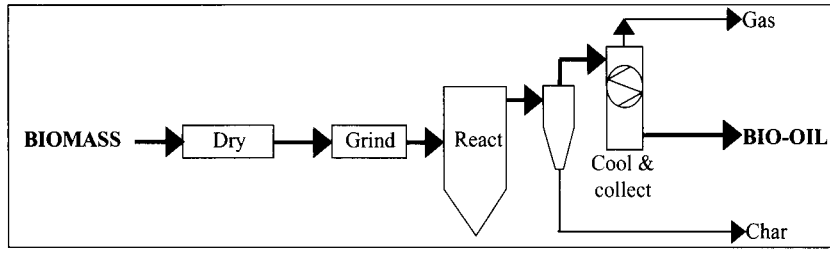


Fig. 33.31. Model for bio-oil production from biomass.

operation and ready scaleup. A typical bubbling fluidized-bed configuration presented in Fig. 33.31 uses the byproduct gas and char to provide the process heat. It also includes the necessary steps of drying the feed to less than 10 percent water to minimize the water in the product liquid oil, and grinding the feed to around 2 mm to supply sufficiently small particles to ensure rapid reaction.

Bio-oil from rapid pyrolysis is usually a dark brown, free-flowing liquid having a distinctive smoky odor. It has significantly different physical and chemical properties compared to the liquid from slow pyrolysis processes, which is more like a tar. Bio-oils are multicomponent mixtures comprised of different size molecules derived primarily from depolymerization and fragmentation reactions of the three key biomass building blocks: cellulose, hemicellulose, and lignin. Therefore, the elemental composition of bio-oil resembles that of biomass rather than that of petroleum oils. Basic properties of bio-oils are shown in Table 33.7. More detail on fuel-related characteristics is provided in the literature.[571]

The single most abundant bio-oil component is water. The other major groups of compounds identified are hydroxyaldehydes, hydroxyketones, sugars, carboxylic acids, and phenolics. Most of the phenolic compounds are present as oligomers having a molecular weight ranging from 900 to 2500. The presence of oxygen in many bio-oil components is the primary reason for differences in properties and behavior from those of fossil hydrocarbon fuels. Because of its high oxygen, bio-oil has an energy density (heating value) that is less than 50 percent of that for conventional fuel oils and it is also immiscible with hydrocarbon fuels. An even more important consequence of the organic oxygen in bio-oil is its limited stability.

Liquid bio-oil can be easily transported and stored. Czernik and Bridgwater reviewed the research on use of bio-oils for heat and power generation, showing that it is possible and usually only requires minor modifications of existing equipment.[550] Bio-oil has been successfully used as boiler fuel and also showed promise for diesel engines and gas turbines.[549,565,571–584] Upgrading bio-oil to a quality for liquid transportation fuels still poses several technical challenges and is not currently economically attractive.[549,564,583,585–588]

Slow Pyrolysis

For many centuries, wood slow-pyrolysis liquids were a major source of chemicals such

TABLE 33.7 Typical Properties of Wood Derived Bio-Oil

Physical Property	*Typical Value*
Moisture content	15–30%
pH	2.5
Specific gravity	1.20
Elemental analysis	
C	56.4%
H	6.2%
O	37.3%
N	0.1%
Ash	0.1%
HHV as produced (depends on moisture)	16–19 MJ/kg
Viscosity (at 40°C and 25% water)	40–100 cp
Solids (char)	1%
Vacuum distillation residue	up to 50%

as methanol, acetic acid, turpentine, and tars. At present, most of these compounds can be produced at a lower cost from other feedstocks derived from natural gas, crude oil, or coal. Though more than 300 compounds have been identified in wood fast pyrolysis oil, their amounts are small. Isolation of specific single compounds is seldom practical or economical, because it usually requires complex separation techniques. Some chemicals produced from the whole bio-oil or by its fractionation are already commercial products. Liquid smoke food flavoring is one example. A few others such as pyrolytic lignin as phenol replacement in resins and bio-oil-based slow-release fertilizer have a chance for short-term commercialization, especially in the context of a bio-refinery based on a fast pyrolysis process. Commercialization of specialty chemicals such as glycolaldehyde or levoglucosan[589] from bio-oil requires more work to develop reliable low-cost separation procedures.

GASIFICATION

Biomass gasification can effectively convert a very heterogeneous material into a consistent gaseous fuel intermediate for heating, industrial process applications, electricity generation, and liquid fuels production. Biomass gasification is a complex thermochemical process that consists of a number of elementary chemical reactions, beginning with the thermal decomposition of a lignocellulosic fuel followed by partial oxidation of the fuel with a gasifying agent, usually air, oxygen, or steam.[590] Volatile matter released as the biomass fuel is heated partially oxidizes to yield the combustion products H_2O and CO_2, plus heat to continue the endothermic gasification process. Water vaporizes and biomass pyrolysis continues as the fuel is heated. Thermal decomposition and partial oxidation of the pyrolysis vapors occur at higher temperatures, and yield a product gas composed of CO, CO_2, H_2O, H_2, CH_4, other gaseous hydrocarbons (including oxygenated hydrocarbons from some processes), tars, char, volatile inorganic constituents, and ash. A generalized reaction describing biomass gasification is as follows.

$$\text{biomass} + O_2\ (\text{or } H_2O) \rightarrow CO,\ CO_2,\ H_2O,\ H_2,\ CH_4 + \text{other hydrocarbons} \rightarrow \text{tar} + \text{char} + \text{ash} \rightarrow HCN + NH_3 + HCl + H_2S + \text{other sulfur gases} \quad (33\text{-}1)$$

The actual amount of CO, CO_2, H_2O, H_2, tars, and hydrocarbons depends on the partial oxidation of the volatile products, as shown in Equation (2).

$$C_nH_m + (n/2 + m/4)\ O_2 \rightarrow nCO + (m/2)\ H_2O \quad (33\text{-}2)$$

The char yield in a gasification process can be optimized to maximize carbon conversion or the char can be combusted to provide heat for the process. Char is partially oxidized or gasified according to the following reactions.

$$C + \tfrac{1}{2}O_2 \rightarrow CO \quad (33\text{-}3)$$

$$C + H_2O \rightarrow CO + H_2 \quad (33\text{-}4)$$

$$C + CO_2 \rightarrow 2CO \text{ (Boudouard reaction)} \quad (33\text{-}5)$$

The gasification product gas composition, particularly the H_2/CO ratio, can be further adjusted by reforming and shift chemistry. Additional hydrogen is formed when CO reacts with excess water vapor according to the water–gas shift reaction.

$$CO + H_2O \leftrightarrow CO_2 + H_2 \quad (33\text{-}6)$$

Reforming the light hydrocarbons and tars formed during biomass gasification also produces hydrogen. Steam reforming and so-called dry or CO_2 reforming occur according to the following reactions and are usually promoted by the use of catalysts.

$$C_nH_m + nH_2O \rightarrow n\ CO + (n + m/2)\ H_2 \quad (33\text{-}7)$$

$$C_nH_m + nCO_2 \rightarrow (2n)\ CO + (m/2)\ H_2 \quad (33\text{-}8)$$

The actual biomass gasification product gas composition depends heavily on the gasification

process, the gasifying agent, and the feedstock composition.[591,592]

Gasifier Reactor Designs

Four primary types of biomass gasification systems have been developed. These include fixed-bed reactors, bubbling fluidized-bed reactors, circulating fluidized-bed reactors, and entrained-flow reactors. Fixed-bed gasifiers can be classified primarily as updraft and downdraft.[593] Updraft gasifiers represent the oldest and simplest gasifiers. The updraft gasifier is a counterflow system where fuel is introduced into the top of the gasifier and flows downward and the gasifying medium is introduced below the grate and flows upwards through the reactor. Feed particle size needs to be controlled to maintain a uniform bed.

Char burns in the combustion zone at the bottom of the reactor, forming CO_2 and steam, which flow upward through the bed. Residual ash is removed from the bottom of the reactor at the grate. Operational problems can be avoided for feeds with low-melting-point ash by carefully controlling the temperature in the combustion zone or with fuel blending or additives to alter the ash chemistry on the grate.

The exothermic combustion reactions supply the energy to drive gasification, pyrolysis, and drying. Above the combustion zone is the reduction zone where CO_2 and H_2O are partially reduced to CO and H_2 according to reactions 4 and 5. The next area of the reactor is the pyrolysis zone where these gases are hot enough to devolatilize the biomass to produce pyrolysis products and residual char. At the top of the reactor the product gases and pyrolytic vapors dry the wet biomass. Product gases exit the gasifier at temperatures (typically 80–100°C) higher than the dew point of the product gas. A wide range of condensable tars and oils is produced in the pyrolysis zone. These can condense in transfer lines at the output of the gasifier. For this reason, updraft gasifiers are usually operated in a close-coupled mode to a furnace or boiler to produce steam or hot water.

In downdraft gasification, the gasifying medium and biomass fuel both flow in the same direction as the solid bed. This design tends to minimize tar and oil production. The fuel and pyrolytic gases and vapors move cocurrently downward through the bed. The pyrolysis products pass through hot char (about 15% of the original feed) into a combustion zone where they mix with air and are thermally cracked and partially oxidized. The oxidizer concentration diminishes downstream of the combustion zone forming a reduction zone. The remaining hot char in the reduction zone reduces some of the combustion products, CO_2 and H_2O, to CO and H_2. The product gas exits the gasifier at fairly high temperature, around 700°C. Downdraft gasification systems typically have low overall thermal efficiency because of heat losses from the product gases, however, tar conversion is high, greater than 99 percent, and is a function of temperature, combustion efficiency, and channeling.

Downdraft and updraft gasifiers have the same general constraints on feed properties. The feed needs to have a fairly uniform particle size with few fines to maintain bed physical properties and minimize channeling. A low-ash feed with a high fusion temperature is also preferred to prevent slagging. Feed moisture content also needs to be less than about 20 percent to maintain the high temperatures required for tar cracking. A variation on the downdraft gasifier is the crossflow gasifier in which air is introduced tangentially at the bottom of the reactor. The principle of operation of the crossflow gasifier is the same as the downdraft gasifier.

Alternative gasifier designs are based on more complex reactor systems to maximize heat transfer and gas–solid interactions by introducing biomass into a moving bed of high-temperature solids.[594] In a gas–solid fluidized bed, a stream of gas passes upwards through a bed of selected free-flowing granular materials such as silica, olivine, limestone, dolomite, or alumina. A gas distribution manifold or series of sparge tubes[595–597] is used to maintain gas velocities high enough to freely circulate the widely separated solid particles

so that the bed resembles a boiling liquid and has the physical properties of a fluid.

Above the bed in a fluidized-bed gasifier, the cross-sectional area of the reactor is increased to produce a disengaging zone where the superficial gas velocity is below fluidization velocity.[598] This allows the entrained sand particles to fall back down and maintain the bed inventory over time. This larger cross-sectional area zone, or freeboard, can be extended to obtain the total desired gas-phase residence time for complete devolatilization. Eroded bed material or fine char and ash particles that escape the reactor can be collected in a cyclone and either returned to the bed or removed from the system.

In a fluidized-bed gasifier, the oxidizer (air, oxygen, or steam) typically doubles as the fluidizing medium. Biomass can be introduced either on top of the bed or through an auger into the bed. In-bed feeding improves the conversion of fine particles that would otherwise be entrained in the fluidizing gas if they were introduced on top of the bed. As biomass is introduced into the bed, the organic pyrolysis vapors are released and partially oxidized in the bed. The exothermic combustion provides the heat to maintain the bed temperature around 800°C and to volatilize additional biomass. Bed temperature is governed by the desire to obtain complete devolatilization (95–99% carbon conversion) versus the need to maintain the bed temperature below the ash-fusion temperature of the biomass ash.

As the gas flow in a bubbling fluidized bed is increased, a turbulent fluidized bed is formed. The gas bubbles become larger, larger voids are formed in the bed, and more solids are entrained in the exit gas flow. A circulating fluidized-bed system results if these solids are collected, separated from the gas, and returned to the bed through a continuous solids circulation loop. A circulating fluidized bed differs from a bubbling fluidized bed in that there is no distinct separation between the dense solids zone and the dilute solids zone. Circulating fluidized-bed densities are about 560 kg/m^3 compared to a bubbling-bed density of 720 kg/m.[599] To achieve the lower bed density, gas rates are increased from the 1.5–3.7 m^3/s of bubbling beds to about 9.1 m^3/s. The residence time of the solids in a circulating fluidized bed is determined by the solids circulation rate, the solids attrition rate, and the collection efficiency of the solids separation device.

Biomass-Gasification Product Gas

Various gasification technologies have been investigated for converting biomass into a gaseous fuel. The energy content of the gasification product gas ranges from 5 MJ/Nm^3 to 15 MJ/Nm^3 and is considered a low-to-medium energy-content gas compared to natural gas (35 MJ/Nm^3).[600,601] The relative amount of CO, CO_2, H_2O, H_2, and hydrocarbons depends on the stoichiometry of the gasification process and the selected gasification medium. The air/fuel ratio in a gasification process generally ranges from 0.2–0.35 and if steam is the gasifying agent, the steam/biomass ratio is around 1. If air is used as the gasifying agent, then roughly half of the product gas is N_2.[602]

Air-blown, or directly heated gasifiers, use the exothermic reaction between oxygen and organics to provide the heat necessary to devolatilize biomass and to convert residual carbon-rich chars. In these directly heated gasifiers, the heat to drive the process is generated internally within the gasifier. Thus, when air is used, the resulting product gas is diluted with nitrogen and typically has a dry-basis calorific value of about 5–6 MJ/Nm^3. The dry-basis calorific value of the product gas can be increased to 13–14 MJ/Nm^3 by using oxygen instead of air. Oxygen production is expensive, however, and its use has only been proposed for direct-heating gasification applications involving the production of synthesis gas where nitrogen is not permitted in downstream synthesis conversion operations. Oxygen typically costs $40 to $60 per Mg and typically is used at the rate of 0.25 to 0.3 Mg/Mg of biomass, a cost equivalent to $10 to $20 per Mg of biomass.[603] Indirectly heated gasifiers accomplish biomass heating and gasification through heat transfer from a hot solid or a heat transfer surface. Because air is not introduced into the gasifier, little nitrogen diluent

is present and a medium calorific gas is produced; dry basis values of 18-20 MJ/Nm^3 are typical.

Gas phase impurities in syngas include NH_3, HCN, other nitrogen-containing gases, H_2S, other sulfur gases, HCl, alkali metals, organic hydrocarbons (tar), and particulates. The concentration of these non-syngas components strongly depends on the feedstock composition. Gasification of biomass containing high nitrogen and sulfur contents yields high levels of NH_3 and H_2S in the syngas stream. HCl concentration in biomass-derived syngas directly correlates with the chlorine content of the feedstock. Alkali metal, mostly potassium, in syngas is related to the alkali content in the biomass ash. Ash particles entrained in syngas affect the alkali metal content in syngas. The concentration of alkali vapors or aerosols in syngas depends on the ash chemistry of the selected biomass feedstock and the temperature of the gasification process.

The organic impurities in syngas range from low-molecular-weight hydrocarbons to high-molecular-weight polynuclear aromatic hydrocarbons. The lower-molecular-weight hydrocarbons can be used as fuel in gas turbines or engines, but are undesirable products in fuel cell applications and methanol synthesis. The higher-molecular-weight hydrocarbons are collectively known as "tar." Tar yields in biomass-derived syngas can range from 0.1 percent (downdraft) to 20 percent (updraft) or greater (pyrolysis) in the product gases.

One issue associated with biomass gasifier tars is how they are defined. More often than not, tar is given an operational definition by those conducting biomass gasification research. An excellent report by Milne, Abatzoglou, and Evans[604] describes in detail the operational definitions of biomass gasification tars as published in the literature and provides a comprehensive survey of tar formation and conversion. For the most part, "tars" are considered to be the condensable fraction of the organic gasification products and are largely aromatic hydrocarbons, including benzene. The diversity in the operational definitions of "tars" usually comes from the variable product gas compositions required for a particular end-use application and how the "tars" are collected and analyzed. Tar sampling protocols are being developed[605–607] to help standardize the way tars are collected; however, these methods are not yet widely established.

Syngas Cleanup and Conditioning

Gas conditioning is a general term for removing the unwanted impurities from biomass-gasification product gas and generally involves an integrated, multistep approach that depends on the end use of the product gas.[608–611] For close-coupled gasifier-combustor systems, no gas cleanup is required. For gas-turbine power generation, the gas has to be free of particulates, tars, sulfur, and chlorine compounds, and of alkali metals to ensure the integrity of the turbine hot section.[612–615] Particulate removal to protect the turbine blades from erosion requires filtration technology, and the removal of tars ensures an even and less luminous combustion process (to avoid radiative heat transfer problems at the turbine). Alkali metal removal avoids deposition and corrosion of the turbine blade materials. Potassium and sodium levels must be reduced to less than 1 part per million to ensure long turbine blade and hot-section lifetimes. The tolerance to alkali metals is a function of the temperature of operation of the turbine inlet section. At very high temperatures (greater than 1350°C) the level has to be less than about 25 parts per billion. The deposition and corrosion problems can be also addressed with turbine blade coatings, and it is likely that both coating and strengthening of components will be undertaken as well, as biomass power technology develops. For internal combustion use, it is necessary to cool the gas to ensure that a sufficient charge of energy can be put into each cylinder. Particular attention has to be given to both tar and particulate contents, to ensure that valves and cylinders are protected. Fuel-cell applications would require the gas to be mainly hydrogen without any significant sulfur or chloride contamination, to

protect the electrodes. For synthesis operations such as methanol and hydrogen production, removal of particulates and contaminants such as H_2S is required to prevent poisoning of downstream catalysts.

Tar removal, conversion, or destruction is seen as one of the greatest technical challenges for the successful development of commercial advanced-gasification technologies.[604] Tars can condense in exit pipes and on particulate filters, leading to blockages and clogged filters. Tars also have varied impacts on other downstream processes. Tars can clog fuel lines and injectors in internal combustion engines. Luminous combustion and erosion from soot formation can occur in pressurized combined-cycle systems where the product gases are burned in a gas turbine. The product gas from an atmospheric-pressure gasification process needs to be compressed before it is burned in a gas turbine and tars can condense in the compressor or in the transfer lines as the product gas cools.

If the end use of the gas requires cooling to near ambient temperatures, it is possible to use wet scrubbing and filtration or other physical removal methods, to remove tars. Wet scrubbing is an effective gas-conditioning process that condenses the tars out of the product gas. This technology is available and can be optimized for tar removal. A disadvantage of wet scrubbing for product gas conditioning is the formation and accumulation of wastewater. This technique does not eliminate tars but merely transfers the problem from the gas phase to the condensed phase. Wastewater minimization and treatment are important considerations when wet scrubbing is used for tar removal. Also, when tar is removed from the product gas stream, its fuel value is lost and the overall efficiency of the integrated gasification process is reduced.

If the end use requires that the product gas remain at high temperature, at or slightly below the gasifier exit temperature, then some method of hot gas cleaning will be needed for tar elimination. Wet scrubbing is still an option, however, there will be a severe thermodynamic penalty from cooling and reheating the conditioned product gas, reducing the overall efficiency of the process. Hot gas conditioning eliminates tars by converting them into desired product gas components, thus retaining their chemical energy in the product gas and avoiding treatment of an additional waste stream. Thermal cracking is a hot gas conditioning option but it requires temperatures higher than typical gasifier exit temperatures (greater than 1100°C) to achieve high conversion efficiencies. Increased temperatures for thermally cracking tars can come from adding oxygen to the process and consuming some of the product gas to provide additional heat. Thermal destruction of tars may also produce soot that is an unwanted impurity in the product gas stream.

An attractive hot gas conditioning method for tar destruction is catalytic steam reforming.[616–621] This technique offers several advantages: (1) catalyst reactor temperatures can be thermally integrated with the gasifier exit temperature, (2) the composition of the product gas can be catalytically adjusted, and (3) steam can be added to the catalyst reactor to ensure complete reforming of tars. Catalytic tar destruction has been studied for several decades[622–624] and a number of reviews have been written on biomass gasification hot gas cleanup.[604,621,625] Numerous catalysts have been tested for tar destruction activity at a broad range of scales. Novel catalyst formulations have been sought to increase the activity and lifetime of tar reforming catalysts. Different approaches for integrating catalytic tar destruction into biomass gasification systems have also been investigated.

Calcined dolomites are the most widely used nonmetallic catalysts for tar conversion in biomass gasification processes.[626–631] They are relatively inexpensive and are considered disposable. However, they are not very robust and quickly undergo attrition in fluidized-bed reactors. Consequently, dolomites find most use in fixed-bed catalytic reactors. Tar conversion efficiency is high when calcined dolomites are operated at high temperatures (900°C) with steam. Olivine, another naturally occurring mineral, has also demonstrated tar conversion activity similar to that of calcined dolomite. Olivine is a much more

robust material than calcined dolomite and has been applied as a primary catalyst to reduce the output tar levels from fluidized-bed biomass gasifiers.

Commercial Ni catalysts are designed for use in fixed-bed applications and are not robust enough for fluidized-bed applications, and therefore are not useful as primary in-bed catalysts. These catalysts, however, have been extensively used for biomass-gasification tar conversion as secondary catalysts in separate fixed-bed reactors operated independently to optimize performance.[632–634] They have high tar-destruction activity with the added advantages of completely reforming methane and of promoting water–gas shift activity that allows the H_2:CO ratio of the product gas to be adjusted. Some studies have also shown that nickel catalyzes the reverse ammonia reaction, thus reducing the amount of NH_3 in gasification product gas.

A limitation of nickel catalyst use for hot gas conditioning of biomass gasification product gases is rapid deactivation, limiting catalyst lifetimes. Ni catalyst deactivation is caused by several factors. Sulfur, chlorine, and alkali metals, that may be present in gasification product gases, act as catalyst poisons. Coke formation on the catalyst surface can be substantial when tar levels in product gases are high. Coke can be removed by regenerating the catalyst, however, repeated high-temperature processing of nickel catalysts can lead to sintering, phase transformations, and volatilization of the nickel. Continued disposal of spent toxic Ni catalysts is not economical and poses an environmental hazard.

Using fixed dolomite guard beds to lower the input tar concentration can extend Ni catalyst lifetimes. Adding various promoters and support modifiers has been demonstrated to improve catalyst lifetime by reducing catalyst deactivation by coke formation, sulfur and chlorine poisoning, and sintering. Several novel, Ni-based catalyst formulations have been developed that show excellent tar reforming activity, improved mechanical properties for fluidized-bed applications, and enhanced lifetimes.

Hot gas conditioning using current or future commercially available catalysts offers the best solution for mitigating biomass gasification tars. Tars are eliminated, methane can be reformed if desired, and the H_2:CO ratio can be adjusted in a single step. The best currently available tar reforming process consists of a calcined-dolomite guard bed followed by a fixed-bed Ni-catalyst reforming reactor operating at about 800°C. Selection of the ideal Ni catalyst is somewhat premature. Commercially available steam reforming catalysts have been demonstrated, but several of the novel research catalysts appear to have the potential of longer lifetimes. This dual-bed hot gas-conditioning concept has been demonstrated and can be used to condition the product gas from any developing gasification process. A proprietary Ni-monolith catalyst has also shown considerable promise for biomass gasification tar destruction.[620]

Biomass-Derived Syngas Utilization

Biomass gasification is a promising thermochemical conversion technique for producing electricity and liquid fuels for power generation because of the high efficiencies projected for integrated-gasification combined-cycle systems. Solid or gasified biomass can be burned as boiler fuel to generate electricity via the steam cycle. Biomass syngas can, however, also be used in diesel, internal combustion, or Stirling engines, in high-efficiency gas turbines, or in high-efficiency fuel cells, all with higher efficiency potential. All of these are suitable for even higher efficiency use with combined heat and power operation. Gasification also enables production of a wide range of fuels and chemicals, such as syngas, hydrogen, and liquid fuels or methanol, ethanol, and Fischer–Tropsch liquids using commercial processes developed in the petrochemical industry for the conversion of methane.

Integrated Gasification Combined Cycle

The integrated gasification combined cycle (IGCC) system has two main components, a

high-efficiency gas turbine and a heat recovery steam generation system. This system uses heat from the turbine exhaust to raise steam and produce electricity in a traditional steam turbine section. Integrated biomass gasification combined cycle systems for electricity production have a number of potential advantages.[612,613,635] Projected process efficiencies are much higher than the direct combustion systems in commercial use today. In a co-generation application, overall system efficiencies can approach 85 percent, thus maximizing the conversion of biomass, a relatively low-energy-density fuel, to heat and power.[636–639] These process efficiencies are comparable to high efficiency large-scale pulverized coal systems, but can be achieved at a smaller scale of operation.

The increased efficiency in IGCC systems translates into environmental benefits because emissions per unit energy produced are lower. This includes CO, NO_x, SO_x, CO_2, and particulates. NO_x and SO_x emissions are inherently lower in biomass IGCC systems because biomass fuels tend to have low N and S contents to start with, and gas cleanup and conditioning in biomass gasification systems removes these impurities before the syngas is combusted in the gas turbine. The same is true for particulates. CO_2 emissions are effectively zero, because biomass is a renewable fuel.

Fuel Cell Applications

Fuel cell systems have the potential to substantially reduce air and water emissions associated with electricity production. In all fuel cell systems, hydrogen is consumed at the anode and water is produced at the cathode. The higher system efficiencies for fuel cells translate into enhanced fuel utilization and therefore reduced CO_2 emissions compared to lower efficiency systems. Fuel cell power plants will be capable of exceeding stringent present and future environmental regulations for particulates, NO_x, and SO_x emissions.

Without considering batteries and other chemical storage devices, there are effectively six types of primary or direct fuel cell technologies currently being developed: alkaline fuel cells (AFC), polymer electrolyte fuel cells (PEFC), a specialized polymer electrolyte fuel cell using methanol as the fuel called the direct methanol fuel cell, phosphoric acid fuel cells (PAFC), molten carbonate fuel cells (MCFC), and solid oxide fuel cells (SOFC).[640–644] These technologies are at various stages of commercialization and individual fuel cell systems have different applications.[643] The high cost of fuel cell systems ($1000–$20,000/kW) is one of the more significant barriers to commercialization.[641]

Integrated biomass gasification fuel cell systems have potential use for distributed and centralized power production. Greater fuel flexibility and greater resistance to impurities makes developing high-temperature MCFC and SOFC systems the most likely technologies for future biomass applications. These systems are currently in various stages of development and demonstration, although molten carbonate fuel cell systems are nearer to commercialization.[645,646] Unlike PAFC systems, MCFCs can tolerate the high concentrations of CO that are produced in biomass gasification.[640,647,648] This eliminates the need for water–gas shifting and selective CO oxidation that would otherwise increase system costs and reduce overall efficiency. The higher operating temperatures of MCFC systems also provide an opportunity for co-generation or waste heat utilization that could improve overall system efficiency to about 85 percent (lower heating value).[649]

Syngas to Liquid Fuels

In its simplest form, syngas is composed of two diatomic molecules, CO and H_2 that provide the building blocks upon which an entire field of fuel science and technology is based.[650–655] Sabatier and Sanderens, who produced methane by passing CO and H_2 over Ni, Fe, and Co catalysts, discovered the synthesis of hydrocarbons from CO hydrogenation in 1902. At about the same time, the first commercial hydrogen from syngas produced from steam methane reforming was commercialized. Haber and Bosch discovered the synthesis of ammonia from H_2 and N_2 in 1910 and the first industrial ammonia synthesis plant was commissioned in 1913. The production of liquid hydrocarbons and

oxygenates from syngas conversion over iron catalysts was discovered in 1923 by Fischer and Tropsch. Variations on this synthesis pathway were soon to follow for the selective production of methanol, mixed alcohols, and isosynthesis products. Another outgrowth of Fischer–Tropsch Synthesis (FTS) was the hydroformylation of olefins discovered in 1938. Many of the syngas conversion processes were developed in Germany during the First and Second World War at a time when natural resources were becoming scarce and alternative routes for hydrogen production, ammonia synthesis, and transportation fuels were a necessity.

Syngas composition, most importantly the H_2/CO ratio, varies as a function of production technology and feedstock. Steam methane reforming yields H_2/CO ratios of three to one whereas coal and biomass gasification yields ratios closer to unity or lower. Conversely, the required properties of the syngas are a function of the synthesis process. Fewer moles of product almost always occur when H_2 and CO are converted to fuels and chemicals. Consequently, syngas conversion processes are more thermodynamically favorable at higher H_2 and CO partial pressures. The optimum pressures depend on the specific synthesis process.

Catalytic syngas conversion processes are exothermic reactions generating large excesses of heat. This highlights the specific need for removing this heat of reaction to carefully control reaction temperatures to maintain optimized process conditions. Maximizing product yields, minimizing side or competing reactions, and maintaining catalyst integrity dictate optimum synthesis reaction temperatures.

Appropriate catalysts are necessary for all fuel and chemical synthesis. The basic concept of a catalytic reaction is that reactants adsorb onto the catalyst surface and rearrange and combine into products that desorb from the surface. One of the fundamental functional differences between various syngas synthesis catalysts is whether the adsorbed CO molecule dissociates on the catalyst surface. For FTS and higher alcohol synthesis, CO dissociation is a necessary reaction condition. For methanol synthesis the CO bond remains intact. Hydrogen has two roles in catalytic syngas synthesis reactions. In addition to being a reactant needed for CO hydrogenation, it is usually used to reduce the metalized synthesis catalysts and activate the metal surface.

Since the genesis of syngas conversion to fuels and chemicals, a tremendous amount of research and development has been devoted to optimizing product yields and process efficiencies. This includes the discovery of catalysts with optimized formulations containing the most active metals in combination with appropriate additives to improve activity and selectivity in a given process. Mechanistic studies have been conducted to interpret the fundamentals of specific conversion processes and measure the kinetic rates of key chemical reactions. Reactor design and engineering is another active research and development area of syngas conversion technology. Temperature control and stability in conversion reactors is a critical process parameter because of the large excess heat of reaction. To optimize commercial synthesis processes, detailed process engineering and integration are used with respect to heat integration and to syngas recycling to improve conversion efficiencies. Given the rich history of syngas conversion and the extensive research and development efforts devoted to this field of study, it is not surprising that a vast amount of literature is available that tracks the scientific and technological advances in syngas chemistry (see the Fischer–Tropsh Archive at www.fischer-tropsch.org). A summary of various catalytic syngas processes, including types of catalyst and reaction conditions, is shown in the "star" diagram in Fig. 33.32.[655] This is by no means a comprehensive list of potential products, but identifies the available processes for utilizing syngas for producing fuels and chemicals.

COMBUSTION

Biomass combustion is the most common and historically oldest method of extracting energy from biomass (other than food) either directly, in the form of heat and light from fire, or indirectly through use of this heat to produce steam

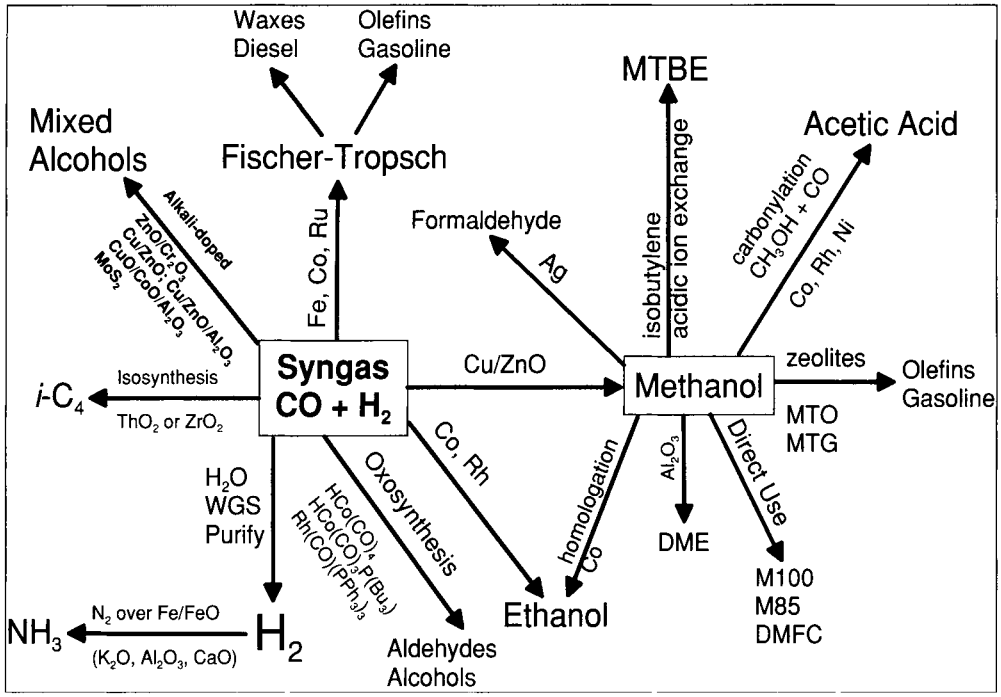


Fig. 33.32. Star diagram showing various high-pressure catalytic processes for converting syngas to fuels and chemicals.

that drives electricity-generating turbines. Biomass is the third largest global source of energy, accounting for 14 percent of annual global energy consumption.[656] The scale of biomass combustion systems ranges from residential heating and cooking applications to small-scale distributed energy production to large-scale centralized power production. Biomass use is very prominent in developing countries, particularly for distributed heat and power and for domestic heat, cooking, and light. Large-scale biomass power plants and industrial biomass utilization for heat and power supplant the residential and commercial biomass heating applications in developed countries.

The impetus for displacing fossil fuels with biomass in heat and power combustion systems is typically to reduce emissions of CO_2, and of NO_x, SO_x, and other air toxics, or to improve utilization of biomass residues and wastes. The threat of increased global warming has subjected the use of fossil fuels to increasing scrutiny in terms of greenhouse gas and pollutant emissions. Renewable and sustainable energy resources such as biomass can be combusted instead of coal to produce heat and power and help reduce the accumulation of greenhouse gases as carbon dioxide is consumed during plant growth. Also, on average, the sulfur content of biomass is lower than coal, so increasing the use of biomass power can reduce sulfur emissions from power production. The agricultural and wood products industries generate large quantities of biomass residues that could provide fuel for electricity production. Increasing the use of these waste biomass fuels could alleviate the burdens of waste disposal in the agricultural and wood products industries.

A number of biomass combustion technologies exist, including grate-fired and fluidized-bed processes.[657] The simplest biomass combustion systems are wood stoves and furnaces. These devices generate hot air that is used for heating residential and commercial buildings. Biomass boiler systems are more fuel flexible than the smaller furnaces and can be scaled to larger size and used to generate hot

water or steam for heat and power applications. Larger-scale biomass boilers include pile burners in which biomass accumulates in a furnace and combustion air is fed from above and below the pile. This relatively simple design is fuel flexible but has low efficiency and poor combustion control, leading to relatively high emissions.

In grate-fired boilers, the biomass is fed in a thin layer, so it is evenly distributed over a sloped stationary, traveling, or vibrating grate. Improved control over the combustion process can be achieved with better carbon conversion. Fluidized-bed combustors are more complex systems, but offer much better control of combustion temperature, improved carbon conversion, and fuel flexibility. Using a boiler to produce both heat and electricity (co-generation) can improve the overall system efficiency to as much as 85 percent. Boiler efficiencies are affected by fuel moisture content, air–fuel ratio, excess air, combustion temperature, and biomass ash content.

Direct combustion to raise steam is used in all of the existing biomass generation plants in the United States today. Biopower is a commercially proven electricity-generating option in the United States and the following statistics for the use of biomass for electricity production can be found in the 2004 Energy Information Agency publications (www.eia.doe.gov). Renewable energy consumption in the United States in 2003 accounted for 6.2 percent of the nation's 98-quad total energy supply. The 2.9 quads of energy from biomass accounted for 47 percent of the renewable energy consumption. Biomass surpassed hydropower as the single largest renewable energy resource for the last several years. Of the total, nearly 90 percent of the biomass energy was consumed in the pulp and paper and forest products industry, three quarters of which was for process heat. Sixty percent of the electricity from biomass is produced from wood or wood wastes with the remaining 40 percent produced from landfill gas, municipal solid waste, and other biomass. Electricity production from biomass is being used and is expected to continue to be used as base load power in the existing electrical distribution system.

Today's biopower capacity is based on mature, direct-combustion boiler/steam turbine technology. The average size of biopower plants is 20 MW (the largest approach 100 MW) and the average efficiency from steam-turbine generators is 17 to 25 percent. The small plant sizes lead to higher capital cost per kilowatt-hour of power produced and the low electrical conversion efficiencies increase sensitivity to fluctuations in feedstock price.[658]

All biomass energy systems suffer from the economic barrier associated with the energy cost of producing, transporting, and preparing the biomass feedstock. Significant progress has been made in this area, but to be truly economically competitive, new feedstocks and methods for their harvest and preparation must be developed. Harvesting, preparation, transportation, and feeding of a variety of biomass feedstocks suitable for power production must be demonstrated, and new methods developed for reducing costs and energy requirements must be verified. This will reduce the delivered cost of feedstock to the energy facility to a level more competitive with fossil fuels.

Technical barriers for biopower applications arise from the variability of biomass fuel compositions. The trace elements in biomass—N, Cl, K, Na, and ash—contribute to operational problems and pollutant emissions in biomass combustion systems.[659–662] The nitrogen content of biomass can vary considerably. Wood and wood wastes tend to have low nitrogen contents because hardwood and softwood trees are relatively slow growing and unfertilized. Agricultural residues and grasses tend to be faster growing and are fertilized and harvested on a yearly basis. Some agricultural residues such as alfalfa and soybeans have value as an animal feed because of their high protein (nitrogen) content. Variability in fuel-bound nitrogen (protein) in biomass leads to varying NO_x emissions in biomass combustion systems.

The chlorine content of biomass is generally a function of the soil conditions it is grown in and the amount of fertilizer applied during growth. Consequently, agricultural residues such as various stovers and straws can have

high Cl levels. Subsequent HCl emissions during combustion can catalyze high- and low-temperature corrosion.

The major technical barrier associated with biomass combustion systems is the formation of tenacious deposits on heat transfer surfaces caused by the unique high-temperature chemistry of biomass ash. Many types of biomass used in combustion systems contain alkali metal species: sodium, potassium, and calcium. The ash content of woody biomass is quite low and not a problem. The ash content of agricultural residues such as wheat straw, rice straw, corn stover, and alfalfa stems can be quite high, on the order of 5 percent or up to 20 percent for rice straw and rice husks. The presence of alkali metals in conjunction with the high silica content of some biomass ashes can lead to molten ash at combustion temperatures.[663–668] In some cases, the K, Si, and Al contents are such that very low melting-point eutectic mixtures can form. The materials can be fluid at combustion temperatures, but form glasslike deposits on colder downstream surfaces such as heat exchanger tubes.

Ash deposition in biomass combustion systems has been the focus of numerous research efforts.[659,669] The basic mechanism for deposit formation in biomass combustion systems starts with the vaporization of alkali metals, usually chlorides, in the combustor. Fly ash particles, which are predominantly silica, impact and stick to boiler tube surfaces. As the flue cools the alkali metal vapors and aerosols quench on the tube surfaces. When the ash chemistry approaches equilibrium on the surface and the deposit becomes molten, the likelihood increases that additional fly ash particles will stick, and deposits grow rapidly. Ash deposits can also accelerate the corrosion or erosion of the heat transfer surfaces. This greatly increases the maintenance requirements of the power plant often causing unscheduled plant interruptions and shutdown.

Co-Firing

One solution to increasing biopower is to build dedicated biomass power plants. An alternative, lower capital cost option for increasing the use of biomass to produce electricity is to co-fire biomass and coal in existing coal-fired power plants. Coal-fired power plants are used to produce the majority of the electricity in the United States. If biomass were co-fired at low percentages in even a small number of coal-fired power plants, the use of biomass for power production would dramatically increase. Co-firing biomass and coal increases the use of sustainable fuels without the need for large capital investments while taking advantage of the high efficiencies obtainable in existing coal-fired power plants. Fuel diversity is another advantage of biomass/coal co-firing. Co-firing reduces the need for a constant supply of biomass that would be required in a dedicated biomass power plant. Co-firing biomass and coal is also a viable way to manage the increasing emissions of greenhouse gases and other pollutants from power-generating facilities.

Biomass and coal have fundamentally different fuel properties that can lead to benefits or deterrents to co-firing. For instance, biomass is a more volatile fuel than coal and has higher oxygen content. Coal, on the other hand, has more fixed carbon than biomass. Wood fuels tend to contain very little ash (on the order of 1% ash or less) and consequently increasing the ratio of wood in biomass/coal blends can reduce the amount of ash that needs to be disposed. A negative aspect of biomass is that it can contain more chlorine than coal. This is particularly true for some grasses, straws, and other agricultural residues.

Better environmental performance at a modest cost is one of the drivers for biomass/coal co-firing in utility boilers.[670–673] Biomass usually has lower sulfur content than coal, so co-firing reduces SO_x emissions because of displacement of sulfur in the fuel blend. Similar reductions are also observed for NO_x emissions, because the nitrogen content of the co-fired biomass fuels is generally lower than the nitrogen content of the coal. Initially, any change in NO_x emissions as a result of blending the biomass and coal can be attributed to changing the amount of nitrogen in the fuel blend.

The addition of biomass has been shown to reduce NO_x emissions in most commercial facilities, usually beyond the reductions

expected because of a lower overall fuel-bound nitrogen content. The high volatiles content of biomass can effectively establish a fuel-rich zone early in the burner flame that can reduce NO_x emissions similar to fuel injection in flue gases (reburning) for NO_x reduction. Adding biomass can also reduce flame temperatures, leading to lower levels of thermal NO_x. The high moisture content of some biomass may also be effective for NO_x reduction at full-scale.

When biomass is co-fired with coal (even in small percentages), the alkali metals in biomass ash can alter the properties of the resulting mixed ash. This could have a significant impact on the coal plant's operating and maintenance costs or even operability. The addition of biomass to a coal-fired power plant can also nullify ash sales contracts for coal flyash. Biomass ash components in feedstocks may also reduce the long-term efficiency and effectiveness of certain (selective catalytic reduction, SCR) systems for the selective catalytic reduction of NO_x.

Fuel preparation and whether to premix the biomass and coal or introduce the two fuels separately into the boiler is another important issue that needs to be addressed for successful implementation of co-firing. Fuel handling of biomass in co-firing systems will need to be demonstrated with a variety of biomass feedstocks—such as switchgrass, willow, and energy cane—to take advantage of lower cost biomass residues and future energy crops.

Biomass co-firing is not a new technology; several utilities have taken advantage of opportunities and have or had been co-firing biomass for many years. One example is the AES (formerly NYSEG) Greenidge Station in Dresden, New York, that began co-firing tests in October 1994.[674] The success of these tests led to the installation of a separate biomass preparation area in the fuel yard in early 1999, to separately feed biomass into the 108-MWe tangentially fired pulverized-coal unit. Another example is Steam Plant #2 at Tacoma Public Utilities' power plant in Tacoma, Washington. This plant was reconfigured in 1991 to co-fire coal, biomass, and refuse-derived fuels on a continuous basis in a 50-MWe atmospheric pressure fluidized-bed combustor. A third example is Northern States Power's King Station in Bayport, Minnesota. From 1987 through 1997, wood residues from the neighboring Anderson Window plant were continuously co-fired at a level of 5 percent (20 tons per hour) in a 550-MWe coal-fired cyclone boiler.[674] Co-firing was suspended at the King Station because Anderson Window found a higher value use for its wood residues, not for technical reasons.

Aside from these longer duration co-firing operations, several utilities have tested biomass/coal co-firing for short durations in utility boilers.[675–679] These various tests sought to systematically determine the impacts of biomass co-firing on such in-furnace parameters as boiler de-rating, boiler efficiency, emissions reductions, changes in fouling and slagging behavior, and corrosion. A number of demonstration projects are currently being funded to add to the knowledge base of utility-scale biomass/coal co-firing.

Several issues clearly remain regarding how blending biomass and coal will affect combustion performance, emissions, fouling and slagging propensities, corrosion, and ash salability. Nevertheless, several utilities have tested biomass/coal co-firing in utility boilers and the Electric Power Research Institute funded a study in 1997 to establish biomass co-firing guidelines.[680]

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